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SYNTHETIC AND BIOCHEMICAL STUDIES

WITH

DEOXYFLUOROMONOSACCHARIDES

BY

DIAGO PHILIP LOPES

A DISSERTATION

Submitted to the Faculty of Graduate Studies through the
Department of Chemistry in Partial Fulfillment
of the Requirements for the Degree of
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Windsor, Ontario
1977

ABSTRACT

Part I

An introduction to the biochemical rationale and the synthesis of fluorocarbohydrates and related compounds is presented.

Attempts to synthesise 4-deoxy-4-fluoro-D-glucose and 4-deoxy-D-glucose by routes amenable to the introduction of ^{14}C are described:

1) The carbon chain extension of 3-deoxy-3-fluoro-D-arabinose (3FA) by Fischer-Kiliani and Sowden-Fischer reactions has been studied. The base-catalysed hydrolysis of the cyanohydrins derived from 3FA gives a mixture of unsaturated hexonic acids. All attempts to convert these acids to the corresponding aldehydes failed.

Nitromethane reacts with 3FA to yield the isomeric 4-deoxy-4-fluoro-1-deoxy-1-nitro-D-hexitols, which crystallise. These latter compounds on strong acid hydrolysis yield 3FA and a compound in poor yields, tentatively identified as 4-deoxy-4-fluoro-D-mannose.

2) By a slightly modified established route, D-galactose is converted into methyl 4-O-methylsulphonyl- α -D-galactopyranoside. Benzylation of the latter compound yields methyl 2,3,6-tri-O-benzyl-4-O-methylsulphonyl- α -D-galactopyranoside (117) in 72% yield. Treatment of the latter compound with tetrabutylammonium fluoride yields methyl 2,3,6-tri-O-benzyl-4-deoxy-4-fluoro- α -D-glucopyranoside (118) which on hydro-

genolysis over Pd/charcoal gives methyl 4-deoxy-4-fluoro- α -D-glucopyranoside (119). The latter compound is converted into the 2,3,6-tri-O-acetate (121) and the position of the fluorine atom in this compound is supported by electron impact mass spectrometric fragmentation. Acid hydrolysis of (119) gives the crystalline free sugar which shows infra red spectrum, melting point, thin layer chromatography (Rf values), and optical rotation etc. consistent with the structure of 4-deoxy-4-fluoro-D-glucose (120). The over-all yields of this alternative synthetic route are sufficient to permit the synthesis of (U 14 C)-4-deoxy-4-fluoro-D-glucose.

3) The synthesis of 4-deoxy-D-glucose from methyl 2,3,6-tri-O-benzoyl-4-O-methylsulphonyl- α -D-galactopyranoside (115) is described. The latter compound (115) undergoes an exchange reaction with sodium iodide in dimethylformamide to yield the corresponding 4-deoxy-4-iodo- α -D-gluco- and galactopyranosides (122) and (123). Hydrolysis of the resulting 4-deoxy-isomer (124) gives crystalline 4-deoxy-D-glucose in sufficiently high yields to allow (U 14 C) synthesis. A similar exchange reaction of methyl 2,3,6-tri-O-benzyl-4-O-methylsulphonyl- α -D-galactopyranoside (117) with sodium iodide yields a mixture of the methyl 4-deoxy-4-iodo-2,3,6-tri-O-benzyl- α -D-gluco- and galactopyranosides (127) and (128).

For biochemical studies the synthesis of 3 H-(C-3)-3-deoxy-3-fluoro- α,β -D-glucose (138) and 3 H-(C-1)-3-deoxy-3-

fluoro-D-glucitol (141) from 1,2,5,6-di-O-isopropylidene- α -D-ribohexofuranos-3-ulose (131) and 3-deoxy-3-fluoro-D-glucose (139) respectively, is described. The over-all radiochemical yields to the fluoroaldose and fluoroalditol are 57% and 46% respectively.

Part II

An introduction to the biochemistry and methodology of D-glucose transport across the human erythrocyte is presented. In particular the systematic use of deoxyfluorosugars and deoxysugars as probes for the hydrogen bonding sites of the sugar to the carrier protein in the human erythrocyte is discussed. The half saturation constant (K_x) of 4-deoxy-4-fluoro-D-glucose and 4-deoxy-D-glucose transport across the human erythrocyte membrane were determined using an established optical method. The K_x values for D-glucose, 4-deoxy-4-fluoro-D-glucose and 4-deoxy-D-glucose are 4.0 mM, 4.6 mM, and 4.5 mM respectively. It is suggested, therefore, that C_4 -OH group of D-glucose is not involved in hydrogen bonding to the carrier protein. These results taken together with those already reported, support a model for stereospecific hydrogen bonds between the oxygen atoms at β - C_1 , C_3 and C_5 of the C_1 -conformation of β -D-glucopyranose and at least three receptor groups on the carrier protein.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to Professor N. F. Taylor, D. Phil., my research advisor, for initiating my interest in the chemistry and biochemistry of fluorinated carbohydrates and for the constant help and encouragement received throughout the course of this work.

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PART 1

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ABBREVIATIONS

| | |
|--------|--|
| 3FA | 3-deoxy-3-fluoro-D-arabinose |
| 3FG | 3-deoxy-3-fluoro-D-glucose |
| 4FG | 4-deoxy-4-fluoro-D-glucose |
| 4FNH | 4-deoxy-4-fluoro-1-deoxy-1-nitro-hexitol |
| g | gram |
| mg | milligram |
| ml | milliliter |
| nm | nanometer |
| m.p. | melting point |
| b.p. | boiling point |
| n.m.r. | proton nuclear magnetic resonance |
| i.c. | infra-red |
| UV | ultra violet |
| LG | leaving group |
| Bz | $-\text{CO}-\text{C}_6\text{H}_5$ |
| Ts | $p\text{-SO}_2\text{-}\phi\text{-CH}_3$ |
| Ms | $-\text{SO}_2\text{-CH}_3$ |
| Tr | triphenylmethyl |
| T.L.C. | thin layer chromatography |
| P.L.C. | Preparative thin layer chromatography |

PART 1

CHAPTER 1

INTRODUCTION

1. General Introduction

Interest in the natural occurrence of fluorinated organic compounds probably arose as a result of the discovery of fluoroacetic acid in the leaves of the South African shrub Dichapetalum cymosum by Marais¹. Since then fluoroacetic acid has been identified in either the seeds or leaves of a large number of plants². Other naturally occurring compounds reported to contain the carbon-fluorine bond are ω -fluoro-oleic acid³, ω -fluorocaproic, ω -fluoromyristic and ω -fluoropalmitic acid⁴. Recently nucleocidin, an antitrypanosomal antibiotic, has been assigned the structure (1). Nucleocidin is an atypical fluorinated carbohydrate, in that fluorine replaces hydrogen. Further interest in the fluorinated compounds was undoubtedly stimulated by the discovery⁶ of 'lethal synthesis' of fluorocitrate from fluoroacetate, which led to the development of new techniques for the synthesis of wide range of fluorinated compounds.

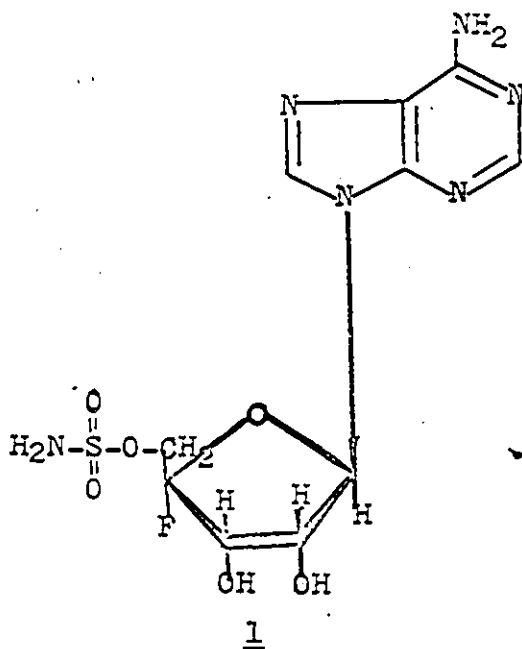
Since a fluorine substituent has a smaller bulk than a hydroxyl group (Table 1) replacement of OH by F in a sugar should not cause marked changes in non-bonded interactions. However, other effects might be expected. For example, whereas a hydroxyl group can act as both hydrogen bond donor and acceptor in interaction with solvent molecules and enzymes, a

TABLE 1

Comparison of the Size and Electronegativity of some Elements⁷.

| Element | Bond Length (CH ₃ -X) (Å ⁰) | Van der waals radius (Å ⁰) | Total | Electro negativity |
|-----------|---|---|-------|-----------------------|
| H | 1.09 | 1.20 | 2.29 | 2.1 |
| F | 1.39 | 1.35 | 2.74 | 4.0 |
| O (in OH) | 1.43 | 1.40 | 2.83 | 3.5 |
| Cl | 1.77 | 1.80 | 3.57 | 3.0 |
| S | 1.82 | 1.85 | 3.67 | 2.5 |

fluorine substituent at best can function solely as a hydrogen bond acceptor. Therefore introduction of fluorine into a metabolite, such as carbohydrate, is a unique way of achieving distinctive modification with minimal disturbance of the overall stereochemistry. The modification may profoundly alter the biochemical behaviour of the fluorosugar in enzyme inhibition or in "lethal synthesis", by which the fluorosugar is partly metabolized into toxic substances⁸. Kinetic, inhibition and nuclear magnetic resonance (n.m.r.) studies of such fluorine-substituted compounds give additional new information about ways in which binding to macromolecules can occur⁹.



4

The usual objective of fluorosugar synthesis is to study the biological activity of such compounds and if this aim is to be fully achieved then the derivatives must be readily available. There is a need, therefore, for short, high-yielding syntheses of fluorosugars. It is convenient to consider the synthesis of fluorosugars from the stand-point of general reactions rather than a particular type of derivative.

II Glycosyl Fluorides (fluoro-alkyl ethers)

The synthetic routes¹⁰ to glycosyl fluorides are well established. They can be prepared by (A) the action of anhydrous HF, or (B) by the action of HF-acetic anhydride, or (C) by the action of AgF, on an appropriate carbohydrate derivative.

IIA The action of anhydrous HF

Because of the anomeric effect, the α -anomeric glycosyl fluorides are thermodynamically more stable than the corresponding β -anomers. Therefore most of the fully acetylated monosaccharides¹⁰ and disaccharides¹¹ afford α -anomeric fluoride by the action of anhydrous HF (Figure 1). Although reaction of the acetylated carbohydrate with hydrogen fluoride normally effects the desired replacement without complications, 1 prolonged treatment sometimes causes deep-seated structural changes^{12,13}, including ring contraction of some hexopyranose derivatives¹⁴.

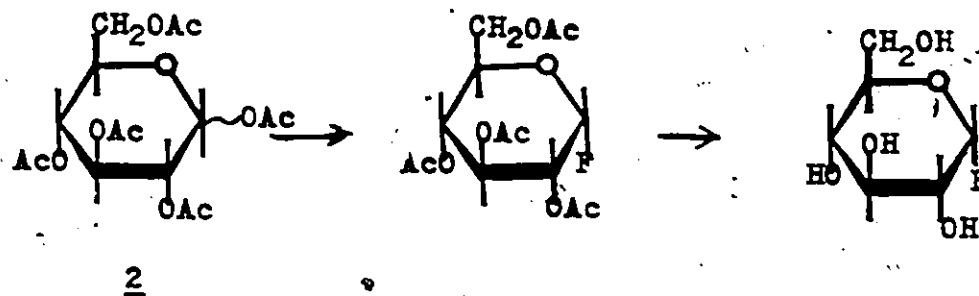


Figure 1. THE ACTION OF HF ON FULLY ACETYLATED MONOSACCHARIDES

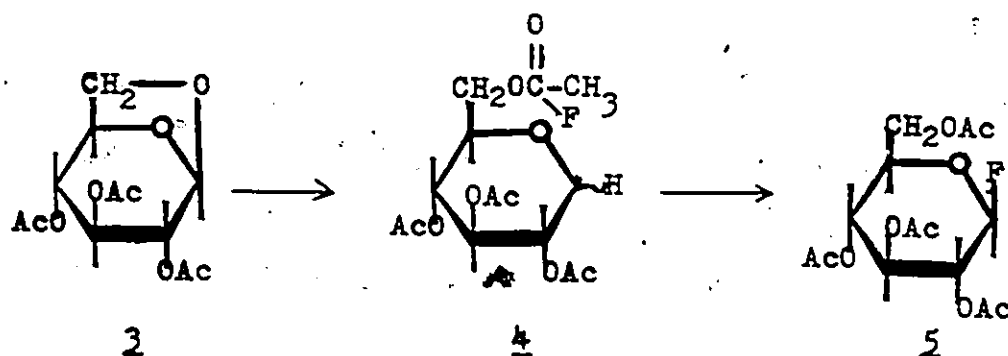


Figure 2. THE ACTION OF HF-ACETIC ANHYDRIDE ON 1,6-ANHYDRO-2,3,6-TRI-O-ACETYL GLUCOPYRANOSE.

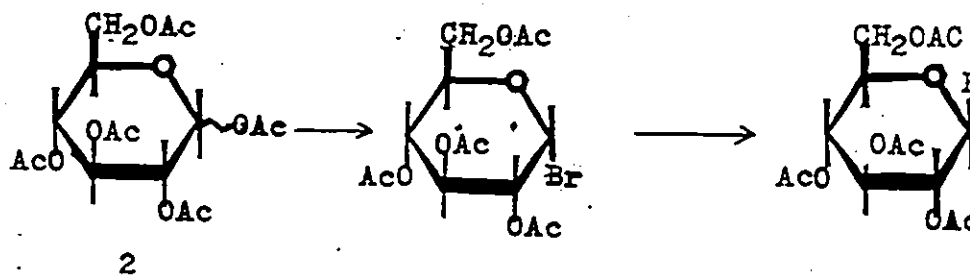


Figure 3. THE ACTION OF AgF ON THE ACETYLATED GLYCOSYL HALIDE.

11B The action of HF-acetic anhydride

This is one of the ways of preparing less stable β -anomeric glycosyl fluorides. Thus the action of HF in acetic anhydride on 1,6-anhydro-2,3,4-tri-O-acetyl- β -D-glucopyranose(3) gave¹⁵ the β -anomeric fluoride (5). The intermediate structure (4) is hypothetical (Figure 2).

11C The action of AgF

Treatment of the acetylated glycosyl halide (bromide or chloride) with AgF normally gives the glycosyl fluoride of opposite configuration (Figure 3); however in some cases^{16,17} retention of configuration at glycosidic carbon has been observed, possibly the unstable anomers which are formed at first which readily isomerize to the more stable anomers.

Unlike any of the other acetylated glycosyl halides, the fluorides may be deacetylated without loss or isomerization of the halide function either with alcoholic ammonia^{18,19} or with a catalytic amount of sodium methoxide in alcohol^{18,20}. In some instances, however, depending on the concentration of base and on the type of substitution at the carbon atoms next to the glycosidic centre, side reactions occur that lead to glycoside (6) or anhydride (7) structure²¹ (Figure 4).

The above mentioned procedures (11A, 11C) have been used to synthesise α - and β -glycosyl fluorides of various 3- and 4-fluorosugars^{21,23} and of 6-deoxy-6-fluoro-D-glucose²⁴.

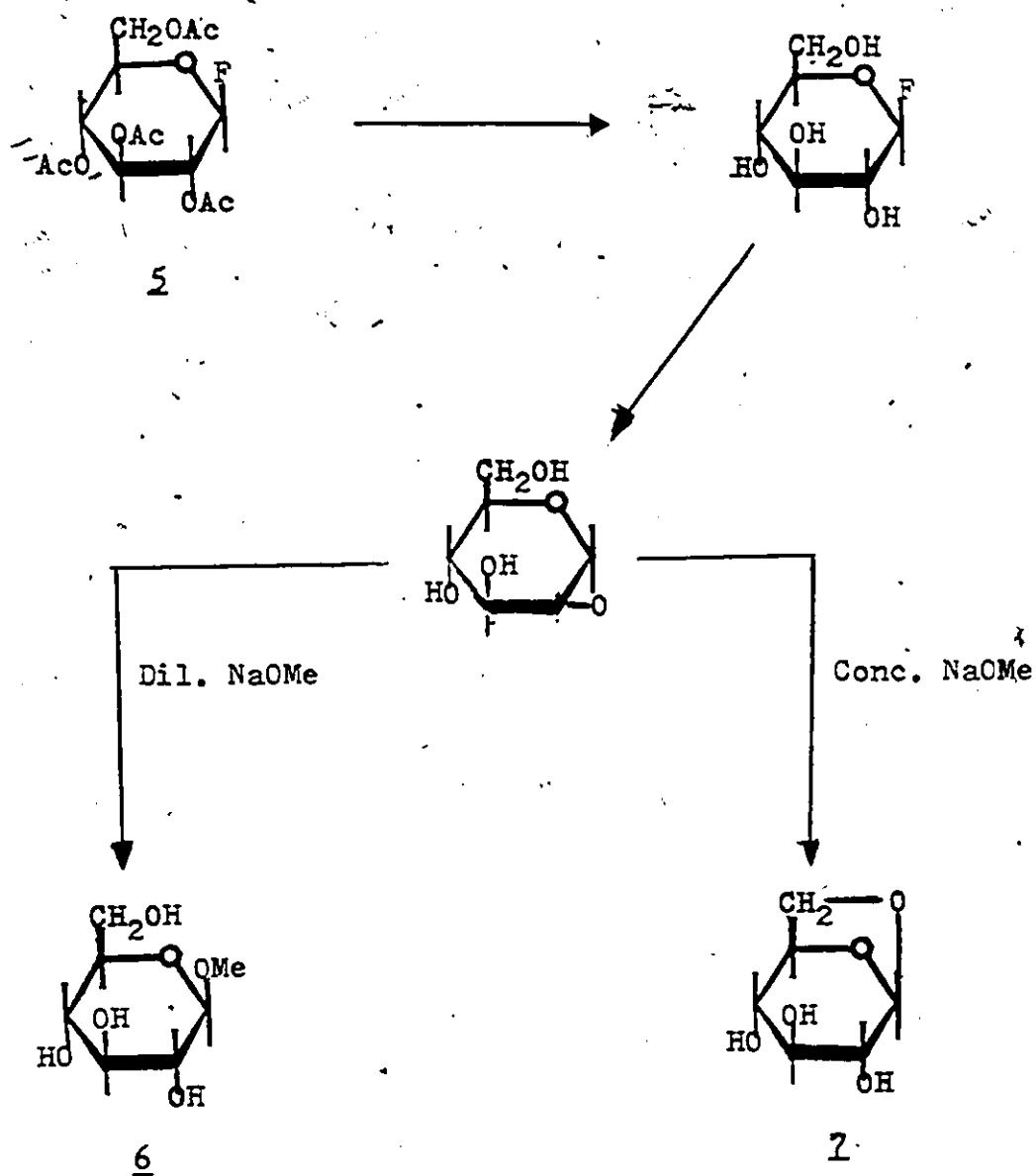


Figure 4. THE HYDROLYSIS OF 2,3,4,6-TETRA-O-ACETYL- β -D-GLUCOSYL HALIDE.

1-1 Synthesis of fluorosugars of the alkyl fluoride type

Three general routes are now available for the synthesis of fluorosugars of the alkyl fluoride type.

- (1) Nucleophilic displacement (usually of sulphate) with fluoride anion.
- (2) Epoxide cleavage reactions.
- (3) Glycal addition reactions.

1-1-1 Nucleophilic displacement with fluoride salts

A substitution reaction, in which the attacking reagent (the nucleophile) brings an electron pair to the substrate, and the leaving group leaves with an electron pair is called a nucleophilic substitution reaction. The substitution reaction may be unimolecular (with a carbonium ion as an intermediate) (S_N1) or bimolecular (S_N2). In the S_N2 reaction mechanism, the nucleophile approaches the substrate 180° away from the leaving group. The reaction is a one step process, with no intermediate. The positions of the atoms and electrons at the top of the curve of the free energy of activation may be represented as (8) (Figure 5). This is the transition state. In molecular orbital terms, the transition state may be described as a point at which the previously unshared orbital of Y overlaps the carbon orbital to about the same extent as does the soon-to-be unshared orbital of X. During the transition state the three nonreacting groups and the central carbon atom are approximately coplanar. The S_N2

reaction, as one can predict by its mechanism, results in Walden inversion when the carbon atom under attack is asymmetric.

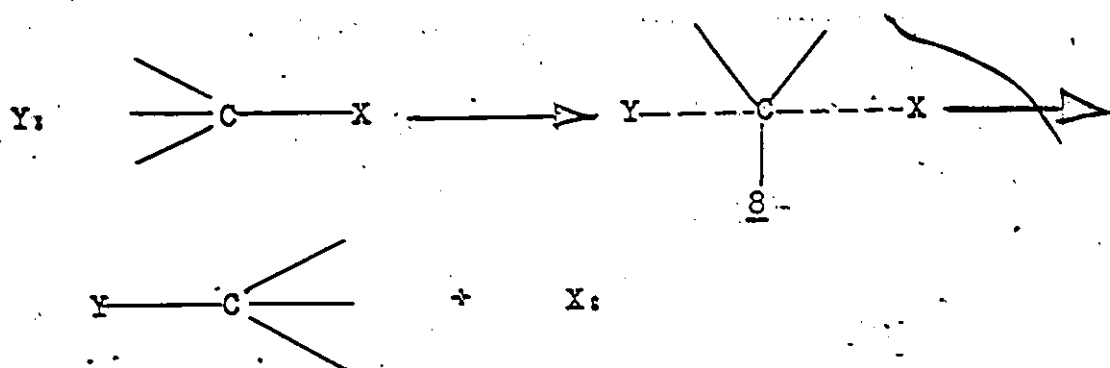


Figure 5 THE S_N2 REACTION MECHANISM.

The sulphonic esters of carbohydrates have proved to be versatile intermediates because they undergo displacement reactions with a variety of nucleophiles⁹⁴, leading to the synthesis of aminosugars⁹⁵, fluorosugars⁵⁴, thiosugars⁹⁶ and deoxysugars⁹⁷. However, displacement of secondary sulphonate groups, particularly those attached to pyranose rings, with charged nucleophiles is not usually possible unless a dipolar, aprotic solvent, such as N,N-dimethylformamide, N-methyl-2-pyrrolidone, hexamethylphosphoric triamide, acetonitrile etc. is employed. The enhancement of reactivity in these solvents was originally attributed to lack of solvation of the nucleophilic anion by the highly basic solvent, thereby increasing

10 ✓

the nucleophilicity⁹⁸. However it has been shown recently that this solvent effect is more likely to be due to greater solvation of the transition state by the solvent, which lowers the activation enthalpy of the reaction⁹⁹. Even in these solvents, the reactivity of a sulphonate group is critically dependent upon its position in the molecule, and it is often possible to predict the reactivity of a sulphonic ester by a consideration of the steric and polar factors affecting the formation of the transition state³⁹.

The direct displacement of a sulphonyloxyl group attached to the carbohydrate moieties normally takes place by an S_N2 mechanism, and therefore the geometry of the transition state involves the generation of two highly polar bonds, one in the process of formation and the other in the process of degeneration. The formation of these polar bonds will be greatly affected by the presence of neighbouring polar substituents, and if these are electronegative in character (e.g., hydroxyl, halogen etc.) the permanent dipole associated with substituent-to-carbon bond should hinder the development of the transition state when the anionic nucleophile is used. Such hindrance will be at a maximum when the dipoles are in the same direction. On the other hand an electropositive substituent ($+NR_3$) would show the reverse effect and should therefore enhance the reaction (Figure 6).

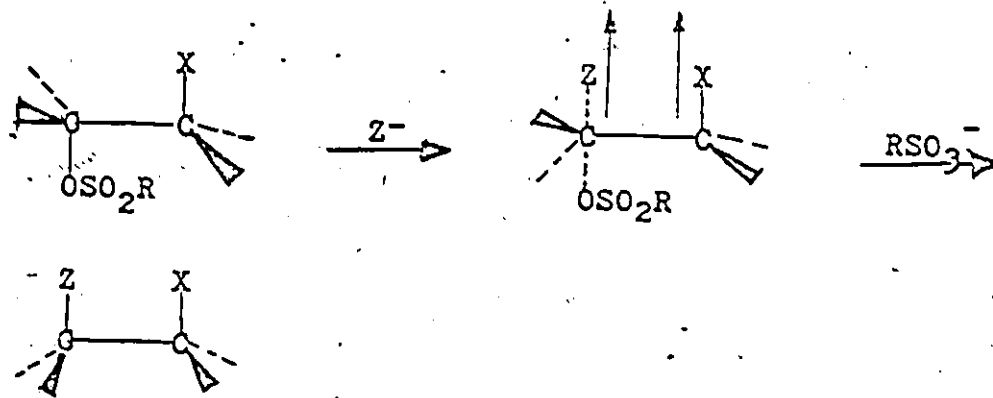


Figure 6. THE S_N2 REACTION OF A CARBOHYDRATE SULPHONATE.

Fluoride displacement reactions are usually effected on sugar sulphonates and although the kinetics have not been investigated, these reactions presumably occur by S_N2 mechanism. It is convenient to consider the nucleophilic displacement reaction of the carbohydrate with fluoride salt under two different categories. (A) Primary sulphonates (B) Secondary sulphonates

III-1A Primary sulphonates

Potassium fluoride is the salt frequently employed for the displacement of primary sulphonates. In the original synthesis of 6-deoxy-6-fluoro-D-glucose²⁵, 3,5-O-benzylidene-1,2-O-isopropylidene-6-O-mesyl- α -D-glucofuranose (9) was

treated with potassium fluoride dihydrate in methanol. In addition to the fluoride (10), the unsaturated compound (11) was formed.

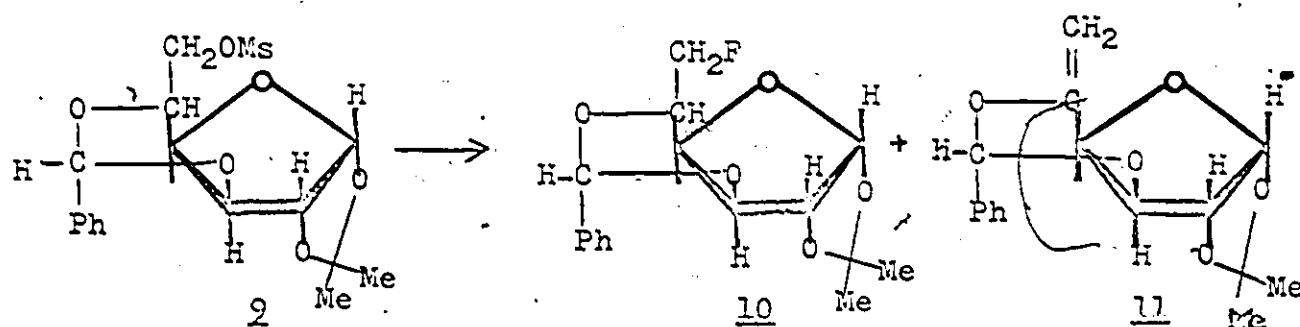


Figure 7. THE ACTION OF POTASSIUM FLUORIDE DIHYDRATE ON PRIMARY SULPHONATE.

When the potassium fluoride-methanol²⁶ was applied to 1,2;3,4-di-O-isopropylidene-6-O-mesyl- α -D-galactopyranoside under vigorous conditions, since nucleophilic displacement of the sulphonate group in this compound was sterically hindered²⁷, the 6-O-methyl ether was formed in addition to the 6-fluoride. The formation of 6-O-alkyl derivative was greatly reduced when ethane-1,2-diol was the reaction solvent with either anhydrous potassium fluoride or its dihydrate. This modified reagent was used in the synthesis of 6-deoxy-6-fluoro-D-galactose²⁸ and 5-deoxy-5-fluoro-D-ribose²⁸ and in an improved synthesis²⁴ of 6-deoxy-6-fluoro-D-glucose. N,N-Dimethylformamide has

also been used as a reaction solvent for fluoride displacement reactions²⁹.

Several sugar derivatives containing a primary fluoride substituent have been obtained by the reaction of the appropriate sulphonate with tetrabutylammonium fluoride (with a variety of protic and aprotic solvents), e.g. 6-deoxy-6-fluoromuramic acid³⁰, 5-deoxy-5-fluoro-D-xylose³¹, 2',5'-dideoxy-5-fluororibonucleosides³², 6,6'-dideoxy-6,6'-difluoro- α,α -trehalose and its galacto analogue³³, 1-deoxy-1-fluoro-D-fructose³⁴, 3,4-di-o-benzyl-6-deoxy-6-fluoro-D-glucal³⁵, 4-o-benzyl-6-deoxy-6-fluoro-3-deoxy-D-glucal³⁵ and 4-o-benzyl-6-deoxy-6-fluoro-2,3-dideoxy-D-glucal³⁵.

The synthesis of 6-deoxy-6-fluoromuramic acid illustrates a further point which must be borne in mind when designing the synthesis of fluorosugars. When the muramic acid derivative (12), was treated with tetrabutylammonium fluoride in butan-2-one, a mixture of the 4,6-diol monoacetates (13) (14) was obtained, presumably because the 4-o-acetate participated in the solvolysis of 6-o-methylsulphonyl group. However, the corresponding 6-tosylate with C₄-OH unblocked (15) underwent smooth fluoride displacement and hydrolysis of the product gave 6-deoxy-6-fluoro-muramic acid (16) (Figure 8).

Direct conversion of alcohols into the corresponding alkyl fluoride had been reported^{36,37} by so called fluoramine reagent, N-(2-chloro-1,1,2-trifluoroethyl)-N,N-diethylamine. However when this reagent was used³⁸ to convert carbohydrate

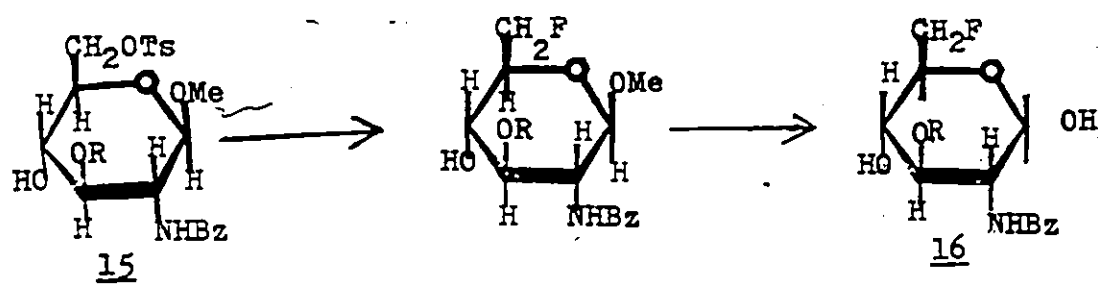
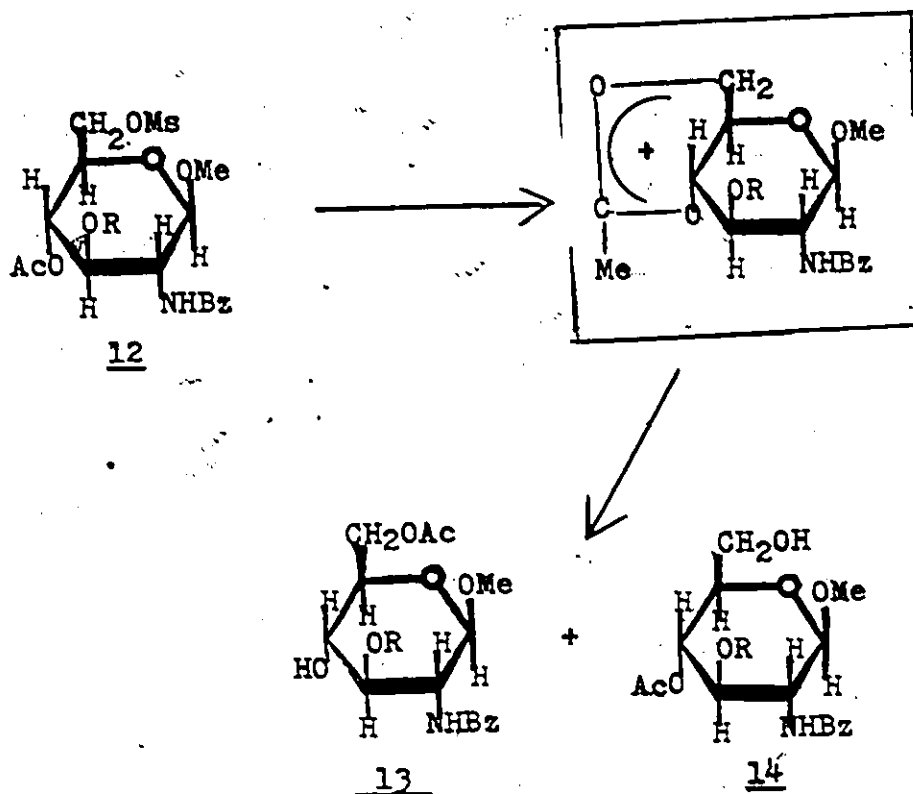


Figure 8. THE SYNTHESIS OF 6-DEOXY-6-FLUORO-MURAMIC ACID.

primary alcohol into the corresponding fluoride, chlorofluoroacetate (17) was isolated as a major product (Figure 9).

Although fluoride displacement of primary sulphonates can usually be effected without difficulty, relatively vigorous reaction conditions are necessary with certain compounds, e.g. 1,2,3,4-di-O-isopropylidene-6-O-mesyl- α -D-galactopyranoside²⁶ and 3-O-benzyl-1,2-O-isopropylidene-5-O-tosyl- α -D-xylofuranose³¹. An explanation for these observations has been advanced³⁹ in terms of steric and polar interactions in the transition state.

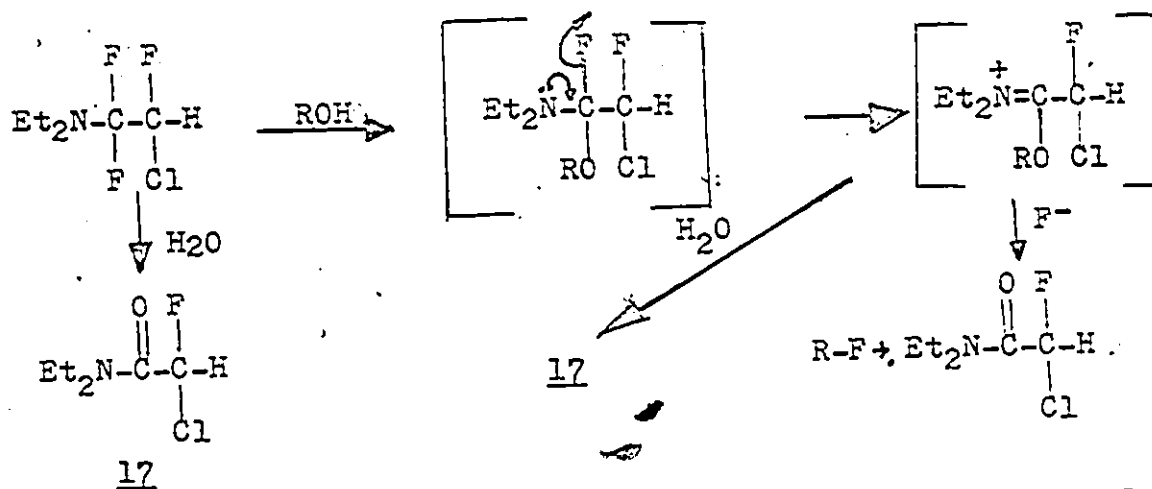


Figure 9. THE ACTION OF FLUORIDE REAGENT ON A CARBOHYDRATE
PRIMARY ALCOHOL (R-OH)

III. 1B Secondary sulphonates

It is convenient to consider the nucleophilic displacement reaction of carbohydrate secondary sulphonates with fluoride salt under two different categories

(a) Furanose derivatives (b) Pyranose derivatives

III-B(a) Furanose derivatives

Fluoride displacement reactions of secondary sulphonates attached to furanoid rings have not been systematically investigated, so that the importance of steric factors and alternative reaction pathways (e.g. elimination) has not been fully defined. One class of furanoid derivative that has been extensively studied includes 1,2-O-isopropylidenehexofuranoses. These compounds contain a cis fused trioxabicyclo-(3,3,0)-octane system and a substituent attached to an exo-position (that is the sulphonate group in 1,2;5,6-di-O-isopropylidene-3-O-tosyl- α -D-glucofuranose (18)) are remarkably resistant to displacement by charged nucleophiles⁴², presumably because the nucleophiles approaching from the endo direction are sterically hindered. On the other hand, endo-sulphonates are readily displaced: thus the conversion of 1,2;5,6-di-O-isopropylidene-3-O-tosyl- α -D-allofuranose (19) into the 3-deoxy-3-fluoro-D-gluco-derivative (21) is readily effected^{43,44} without any elimination reaction, with tetrabutylammonium fluoride in acetonitrile. However, in the corresponding gulo-compound (20) elimination

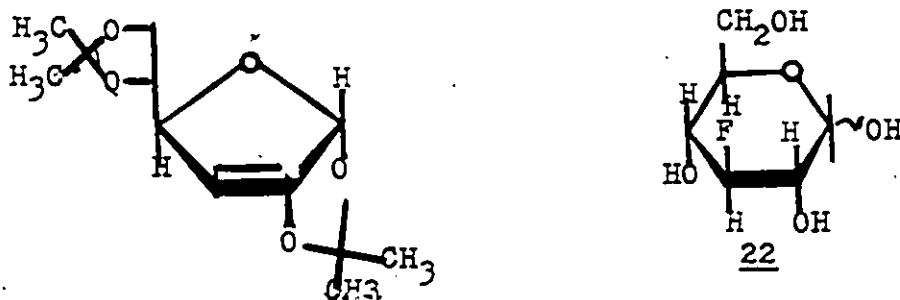
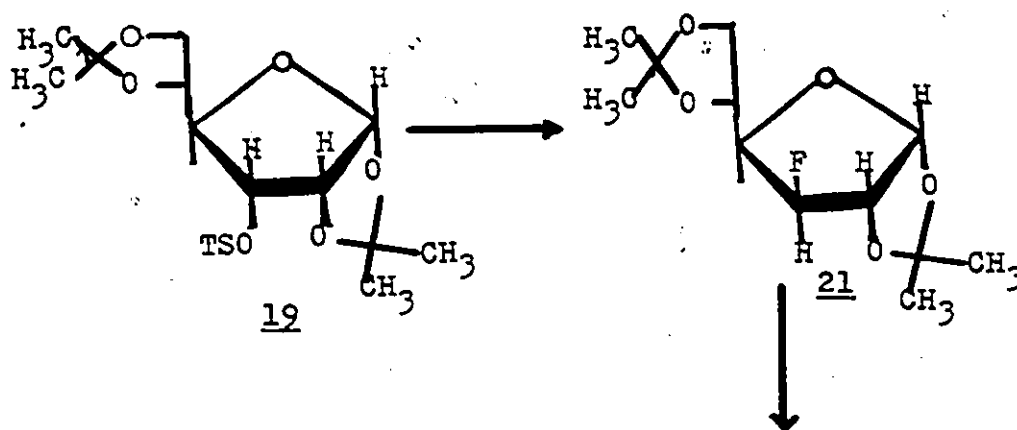
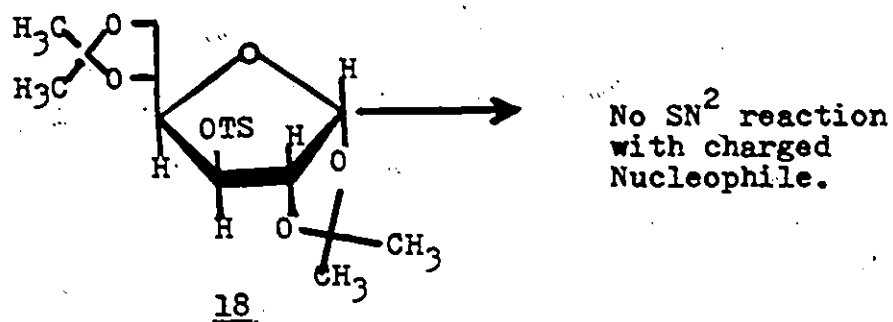
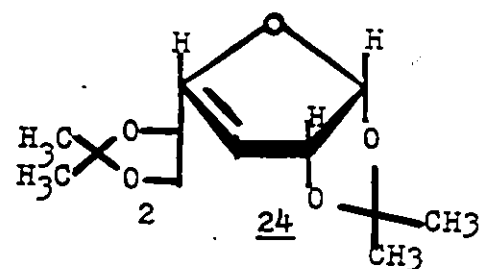
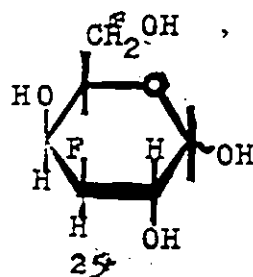
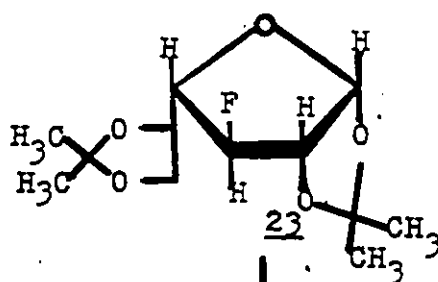
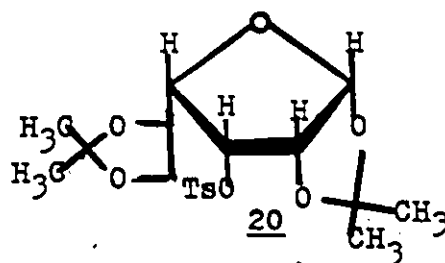


Figure 10. FLUORIDE DISPLACEMENT REACTION OF FURANOID
SECONDARY SULPHONATE.

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(to give 24) occurs to approximately the same extent as fluoride displacement^{45,46} to yield the 3-deoxy-3-fluoro-D-galacto derivative (23). Acid hydrolysis of compound (21) and (23) yield 3-deoxy-3-fluoro-D-glucose^{42,43} (22) and 3-deoxy-3-fluoro-D-galactose^{45,46} (25) respectively (Figure 10).

A third example in this category involves the treatment of 1,2;5,6-di-O-isopropylidene-3-O-tosyl- β -L-talofuranose with tetrabutylammonium fluoride in acetonitrile to yield 3-deoxy-3-fluoro-1,2;5,6-di-O-isopropylidene- β -L-idofuranose^{47,48}. However a more convenient entry into the 3-fluoro-L-ido (27) series is afforded by the ready availability of the 3-deoxy-3-fluoro-D-glucose derivative (21). The relevant reaction sequence, which is shown in Figure 11 together with that for 3,5-dideoxy-3,5-difluoro-D-xylofuranose^{48,49} illustrates how a readily available fluorosugar derivative can serve as a starting point for a new synthesis.

III-1B(b) Pyranose derivatives

The steric and polar factors which influence nucleophilic displacement reactions of pyranose secondary sulphonates are reviewed³⁹ by A. C. Richardson. It is appropriate for the present work to consider the reactivity of pyranose 4-sulphonates.

The reactivity of pyranoside 4-sulphonates

The development of an S_N2 transition state at C_4 is not

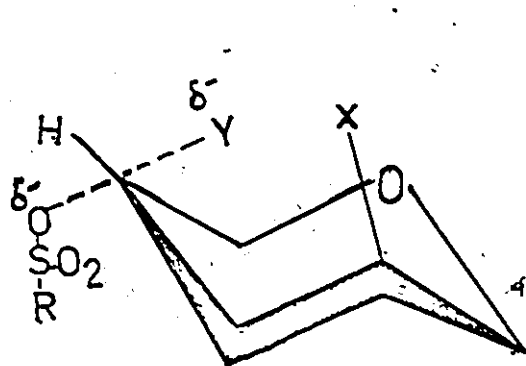
greatly affected by the dipole associated with the anomeric group but is mainly affected by steric and polar factors from other groups in the ring. There are two factors which seem to affect displacement at C₄ position.

(a) The β -trans-axial substituent effect

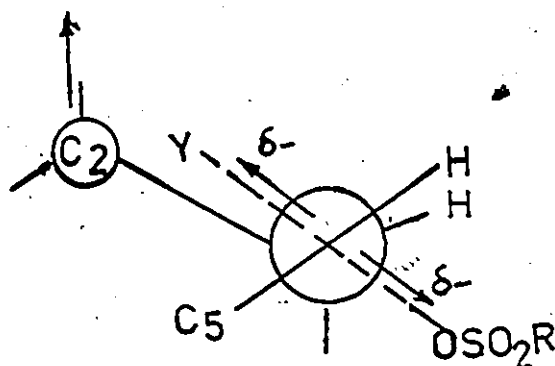
(b) effect of a vicinal axial substituent

(a) The β -trans-axial substituent effect

When a β -trans-axial substituent is present with respect to a sulphonyloxy group, its displacement is impaired³⁹. The β -trans-axial effect is probably due to steric (and perhaps polar) factors in the transition state, and can be visualised with a pyranoid equatorial 4-sulphonate which has an axial group at the 2-position. The steric and polar clash in the transition state (29) is best seen by viewing along the C₄-C₃ bond (30). There is considerable steric interaction between the incoming nucleophile (Y:) and the 2-substituent (X), giving rise to a transition state of high energy. It is also possible that there is some dipolar repulsion between the upper, polar bond of the transition state and the dipole associated with the C₂-X bond which are almost parallel with their negative ends converging. This is supported by the fact that methyl 2,3,6-tri-O-benzoyl-4-O-methanesulphonyl- α -D-mannopyranoside (axial 2-benzoate) is resistant to replacement¹⁰⁰, whereas the corresponding glucopyranoside reacts^{101,102}.



29



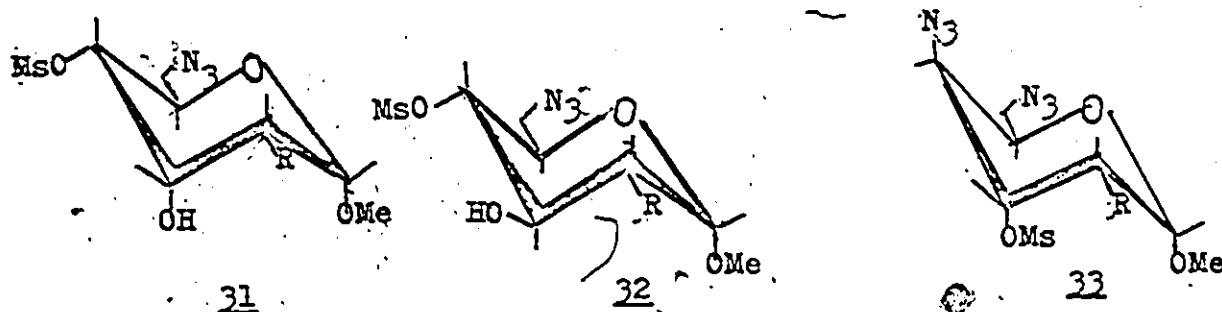
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(b) The effect of a vicinal axial substituents

The presence of a vicinal axial group cis or trans to the sulphonyloxy group on a pyranoid ring, inhibits replacement of that group^{101, 102, 103}. This was interpreted as being due to an increase in steric interaction in the transition state of the reaction, since the departing sulphonate group moves closer to the neighbouring axial group³⁹. This is supported by the fact that compounds (31) and (33) having vicinal axial groups are resistant to azide or benzoate ions in hexamethylphosphoric triamide, conditions under which the corresponding glucopyranoside derivative (32) reacts readily³⁹. 1,6-anhydro-2,3-O-isopropylidene-4-O-methylsulphonyl- β -D-mannopyranose

and -talopyranose are resistant¹⁰⁴ to azide ion under forcing conditions, and this lack of reactivity may be ascribed, at least in part, to the presence of an adjacent, axial, electronegative substituent at C₃.

R = NHBz



The examples of nucleophilic displacement of pyranose secondary sulphonates in the field of fluorosugars are the synthesis of 4-deoxy-4-fluoro-D-glucose⁵⁰ and 4-deoxy-4-fluoro-D-galactose⁵¹.

III-1B(c) Septanose derivatives

Treatment of D-glucose with acetone-methanol-hydrogen chloride gives mainly methyl 2,3,4,5-di-O-isopropylidene- α , β -D-glucoseptanoside^{52,53} and makes these hitherto inaccessible compounds readily available. The α -anomer (34) can be readily converted⁵⁴ into methyl 5-O-benzyl-3-4-O-isopropylidene-2-O-

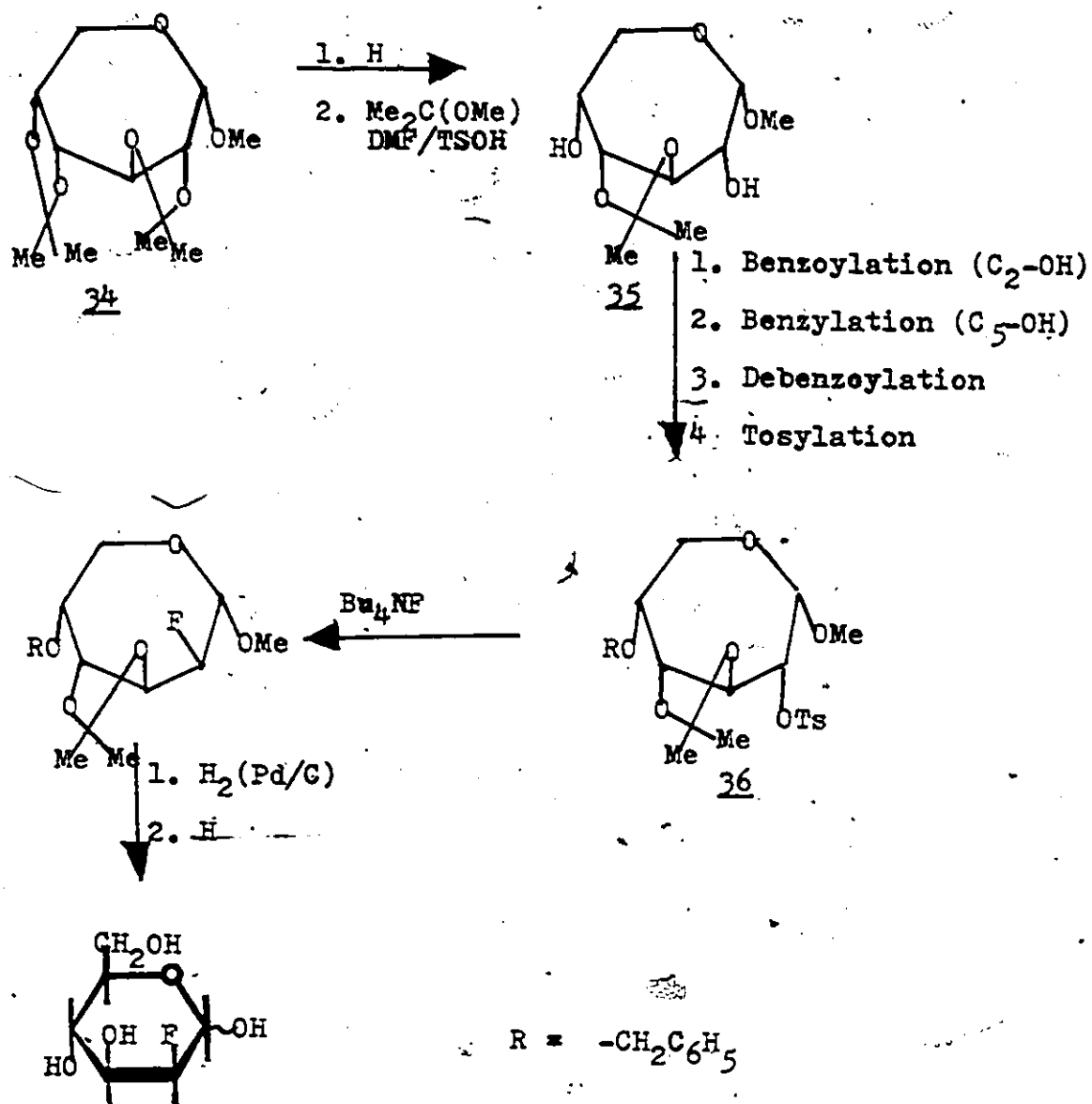


Figure 12. THE SYNTHESIS OF 2-DEOXY-2-FLUORO-D-ARABINOSE.

tosyl- α -D-glucoseptanoside (35) which is converted by tetrabutylammonium fluoride-acetonitrile into the corresponding 2-deoxy-2-fluoro-D-mannoseptanose derivative (36) (Figure 12). The susceptibility of the D-glucoseptanose-2-sulphonate derivative (35) to the nucleophilic displacement is in marked contrast³⁹ to the resistance of pyranoid 2-sulphonates to displacement with charged nucleophiles. The availability of septanose derivatives adds a new parameter in carbohydrate synthesis.

III-1B(d) Acyclic derivatives

Only one fluoride displacement of an acyclic carbohydrate secondary sulphonate has been reported. Treatment^{55,56} of 2-O-tosyl-1,3-di-O-trityl glycerol (37) with tetrabutylammonium fluoride-acetonitrile gave a high yield of the 2-fluoro derivative. Detritylation then gave 2-deoxy-2-fluoroglycerol (38).

1-deoxy-1-fluoro-D-glycerol (40) was obtained^{55,56} via a fluoride displacement reaction on 2,3-O-isopropylidene-1-O-tosyl-D-glycerol (39). When the 2,3-ditosylate of the D-isomer (41) was subjected to a benzoate displacement reaction and the product debenzoylated, 1-deoxy-1-fluoro-L-glycerol was produced (42) (Figure 13).

III-2 Epoxide cleavage reactions

Carbohydrate epoxides in which a molecule of water has been removed from a pair of adjacent carbon atoms, leaving

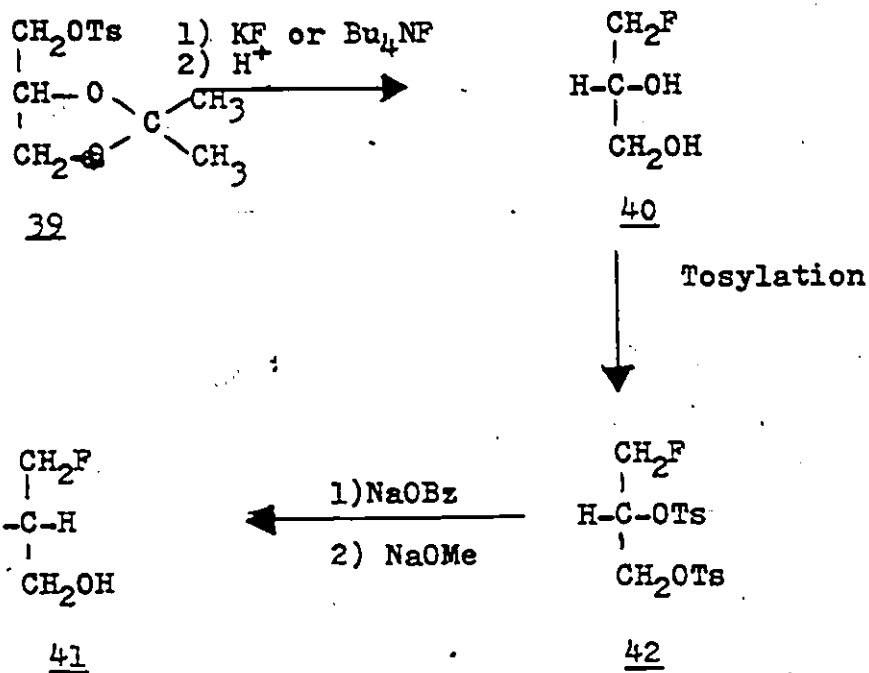
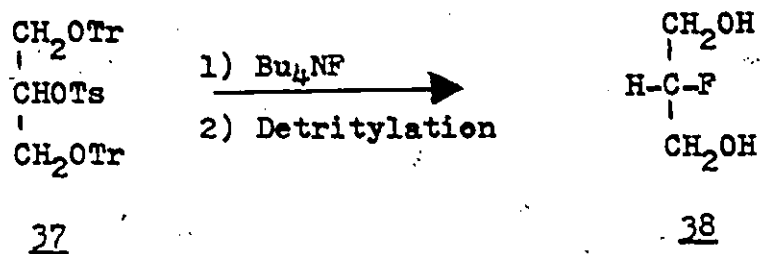


Figure 13 THE SYNTHESIS OF ACYCLIC FLUOROCARBOHYDRATES.

them with oxygen bridged, were first isolated⁵⁷ in 1933. Nucleophilic scission of epoxide occurs with both acidic and basic reagents, but is generally more rapid with acidic reagents, where initial protonation of the oxygen gives a charged structure, providing a driving force to facilitate electron flow during the rate-determining attack of the nucleophile. Apart from this, acidic and basic scission are similar, and are shown in Figure 14.

Thus, nucleophilic scission of epoxide leads to two possible products in each of which the carbon atom carrying nucleophile has undergone Walden inversion. In the monocyclic carbohydrates, pyranose epoxides possess flexible conformations, which can be regarded as 'half-chair' forms (Figure 15). Both conformers may react by axial attack. The product can then change into the more stable conformers. Studies on a carbohydrate epoxide in which the conformation is made rigid, (e.g. 1,6-anhydro bridge⁵⁸) confirmed that the ring opening takes place predominantly to give trans-diaxial products, although equatorial opening does occur to a limited extent.

Early difficulty of carbohydrate epoxide opening was partially overcome by Taylor et al⁵⁹ by use of anhydrous hydrogen fluoride in dioxane. The use of dioxane as a solvent results in the formation fairly stable oxonium ions, which weaken the H-F hydrogen bonding, thereby enhancing nucleophilicity of the fluoride. Considerable success in carbohydrate epoxide scission has followed the introduction of potassium

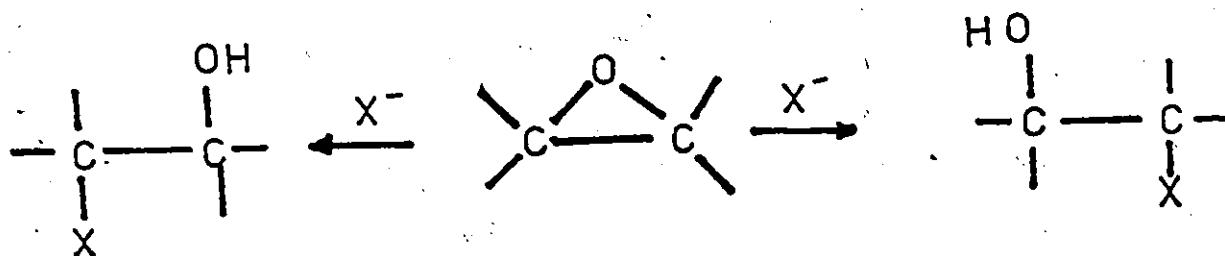


Figure 14. THE MONOCYCLIC CARBOHYDRATE EPOXIDE SCISSION.

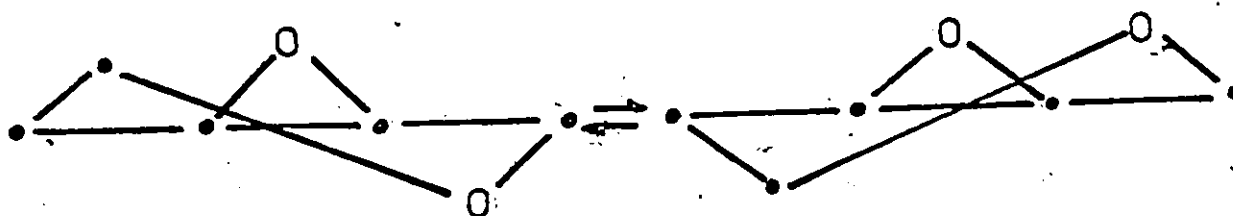


Figure. 15. THE MONOCYCLIC CARBOHYDRATE EPOXIDE.

hydrogen fluoride⁶⁰ (KHF_2) in ethane-1,2-diol. The first secondary fluorosugars in both pentose and the hexose series were obtained by the epoxide route as shown in Figure 16. In each of these synthetic methods the epoxide of a monocyyclic carbohydrate was used. Of particular interest are the anomeric methyl 2,3-anhydro-5-O-benzyl-D-ribo-furanosides (43,44). The epoxide cleavage with KHF_2 of the α -anomer (44) yields⁶¹

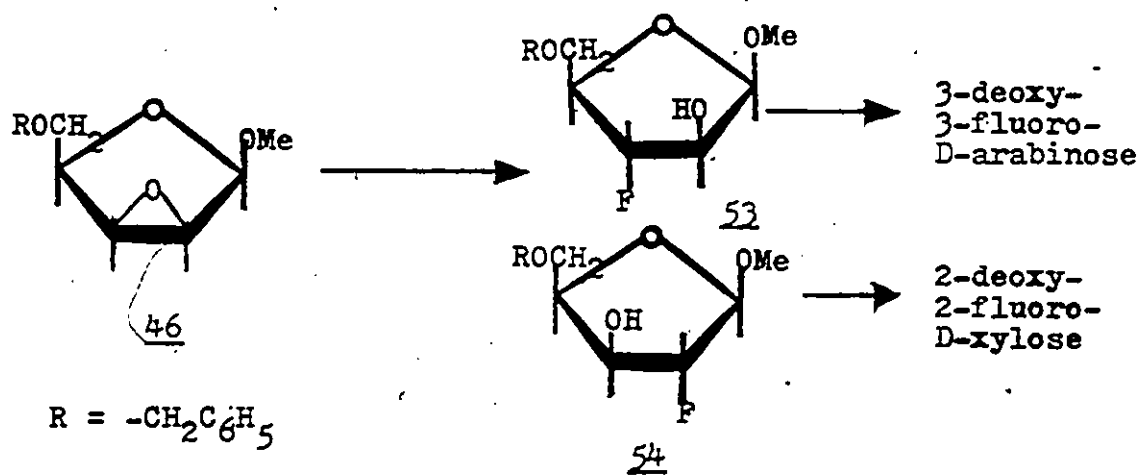
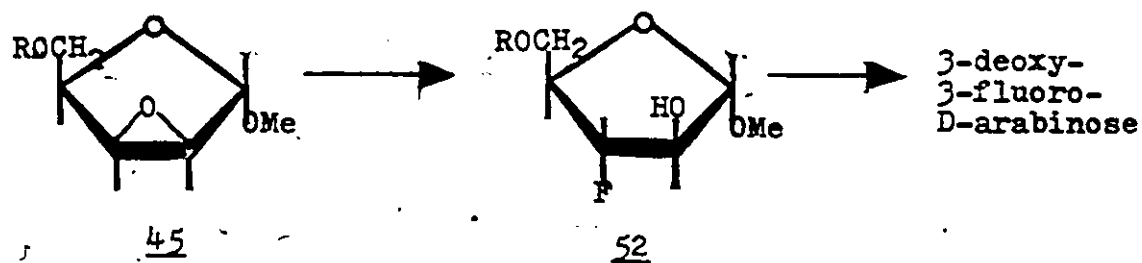
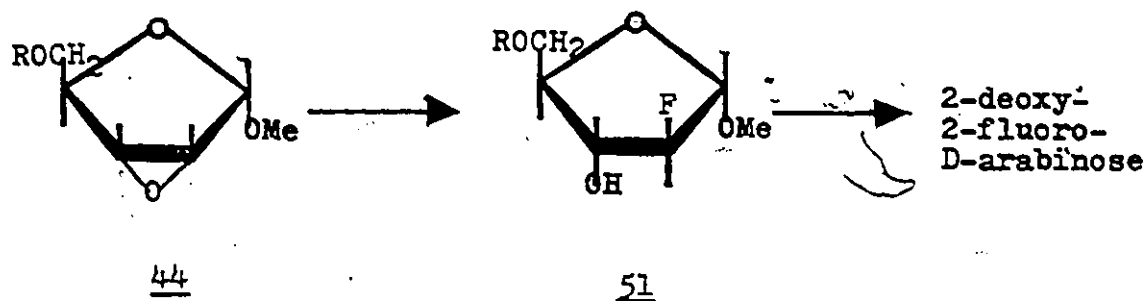
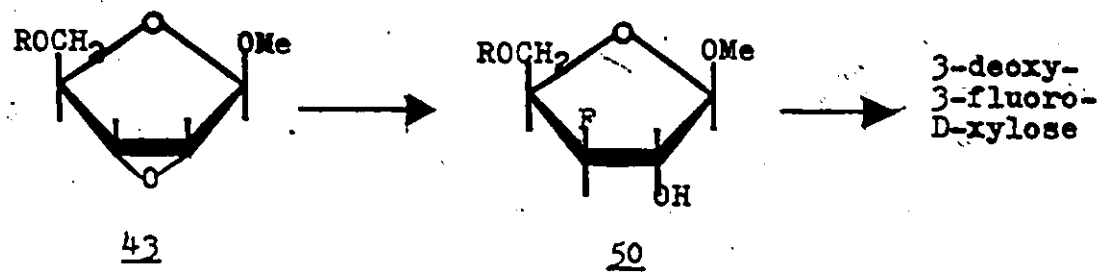
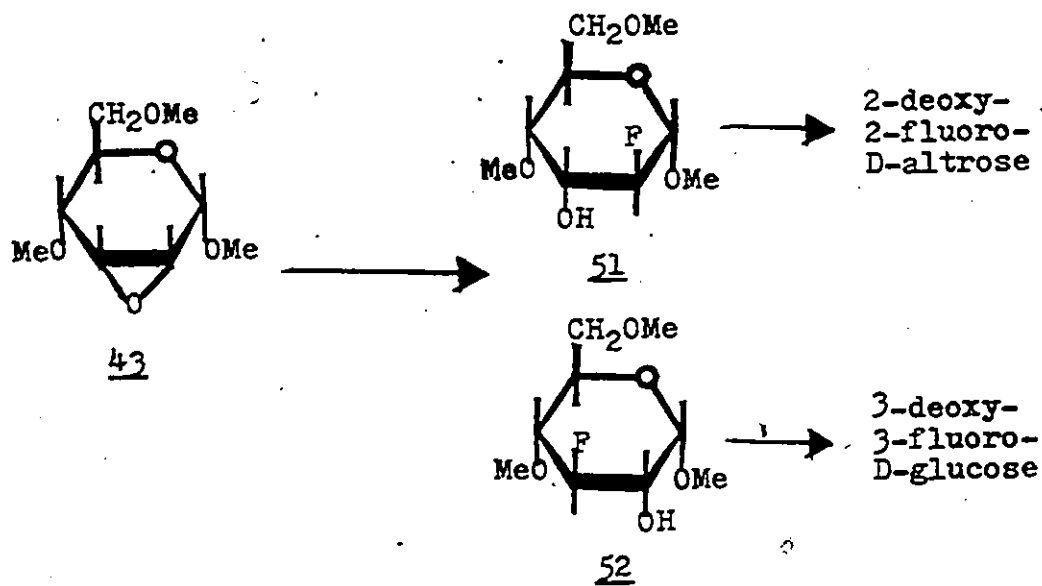
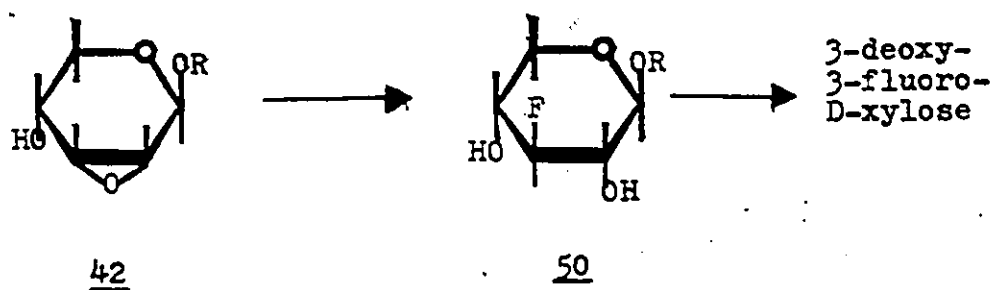
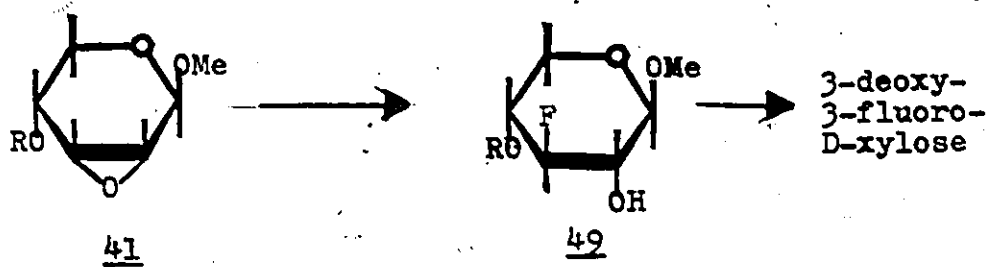


Figure 16. THE EPOXIDE OPENING OF PENTOSE AND HEXOSE BY
 KHF_2/HF .

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mainly a 2-fluoro-D-arabinose derivative (51); the β -anomer (43) under similar conditions gave⁶² preponderantly the 3-deoxy-3-fluoro-D-xylose derivative (50). Anomeric methyl-2,3-anhydro-5-O-benzyl- α,β -D-lyxofuranosides (45,46) have also been studied^{61,63} with KHF_2/NaF treatment. The epoxide cleavage with KHF_2 of the α -anomer (45) gave⁶³ mainly 3-deoxy-3-fluoro-D-arabinose derivative (52); but the corresponding β -anomer under similar conditions gave⁶³ mixture of arabinose (53) and xylose (54) derivatives.

In a pyranose system^{60,63} compounds (47) and (48) which differ only in the blocking group at $\text{C}_1\text{-OH}$ and $\text{C}_4\text{-OH}$ gave the same product (3-deoxy-3-fluoro-D-xylose derivative) by epoxide cleavage with KHF_2 . In the hexose series studied by Johnston and Lindberg⁶⁴, methyl 2,3-anhydro-4-di-O-methyl- α -D-allopyranoside (49) with hydrogen tetrafluoridoborate in HF gave the 2-deoxy-2-fluoro-D-altrose derivative (57) (major) and 3-deoxy-3-fluoro-D-glucose derivative (58). However under their experimental conditions, a small amount of 2-deoxy-2-fluoroaltrosyl- α -fluoride was produced by displacement reaction of -OMe group by fluoride.

More recent work has utilized 3,4-epoxide derived from 1,6-anhydro- β -D-glucopyranose^{65,66,67,68}. The bridged bicyclic system in this compound greatly reduces the flexibility of the pyranose ring, so that epoxide cleavage is usually stereospecific and predictable giving trans-diaxial product.

A type of fluoride displacement unique to the nucleoside field involved the cleavage of cyclonucleosides with hydrogen fluoride. Two examples have been reported involving O^2 , $2'$ -cyclo-1-(β -D-arabinofuranosyl)thymine and uracil⁶⁹ (59) and O^2 -3'-cyclo-1-(2'-deoxy- β -D-threo-pentofuranosyl) thymine⁷⁰ (60) (Figure 17). These reactions which are apparently acid catalyzed, are analogous to those involving epoxide cleavage.

III-3 Glycal addition reactions

Glycals (enol ethers) constitute a class of carbohydrate derivative which are of particular value for the synthesis of 2-substituted derivatives. In ionic, 1,2 addition reactions it is usually C-2 of a glycal which behaves as a nucleophilic centre, so that reaction proceeds along the pathway as shown in Figure 18. Thus the mixed halogen IF (generated from silver fluoride and iodine), which can be regarded as a source of electrophilic iodine and nucleophilic fluoride, adds preponderantly trans to 3,4,6-tri-O-acetyl-D-glucal (61), to give⁷¹ (62), (63) and (64).

This reaction pathway has been elegantly utilized in the synthesis⁷² of antibiotic nucleocidin (1). Treatment of exomethylene nucleoside (65) with iodine-silver fluoride gave a mixture of the epimeric 5'-deoxy-4'-fluoro-5'-iodonucleosides; the ratio of β -D-ribo and α -L-lyxo isomers was markedly solvent dependent. The β -D-ribo isomer was separated and converted into nucleocidin as shown in the Figure 19.

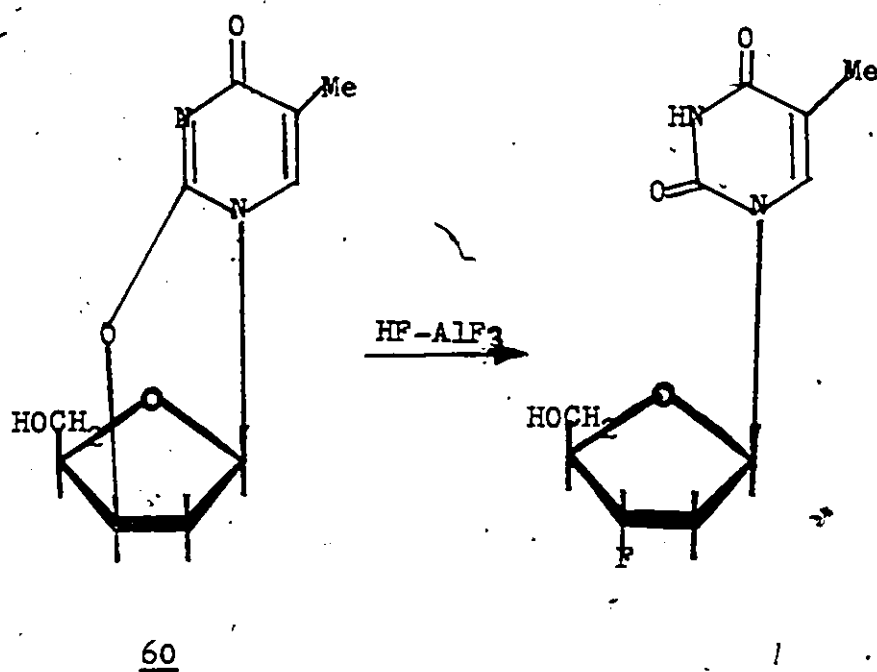
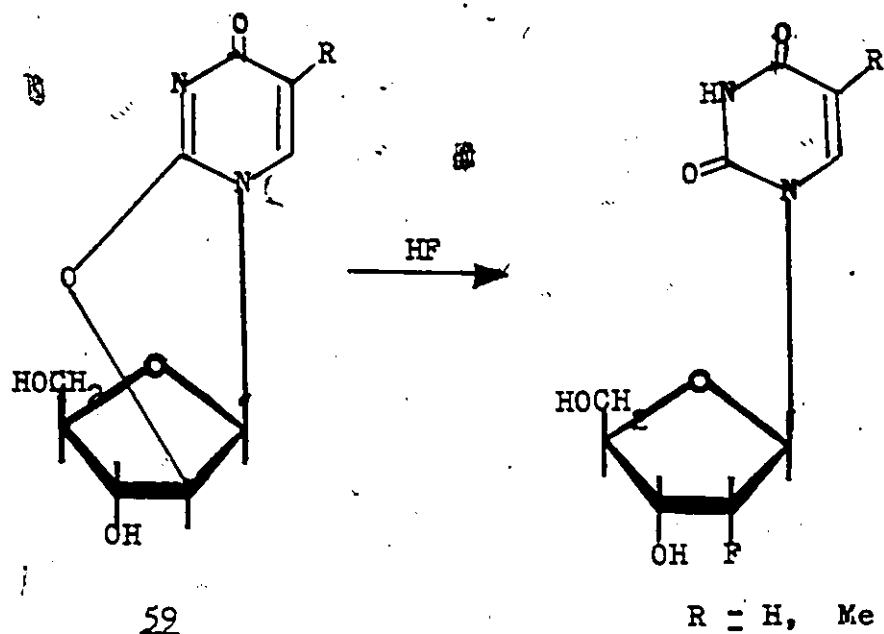


Figure 17. THE EPOXIDE TYPE SCISSION OF CYCLONUCLEOSIDES.

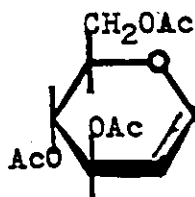
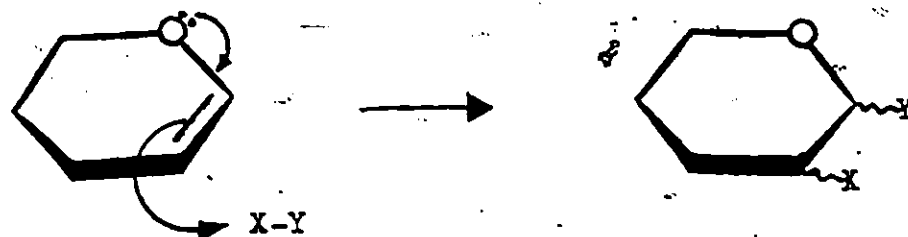
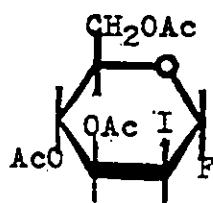
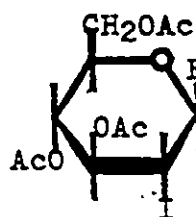
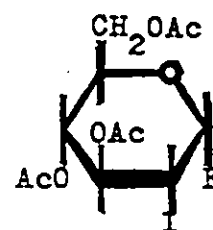
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Figure 18. THE ADDITION OF MIXED HALOGEN ACROSS A GLYCAL DOUBLE BOND.

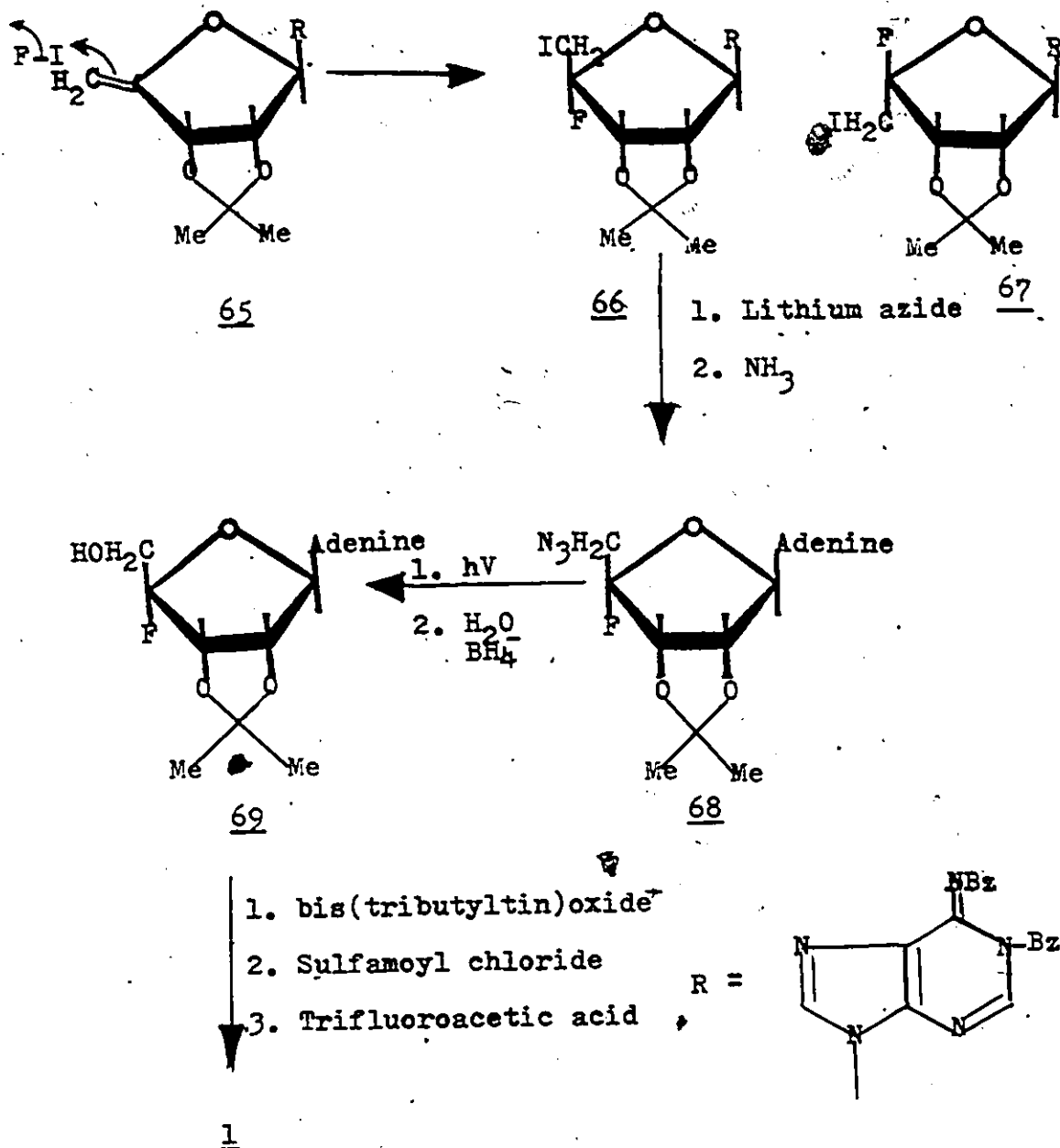


Figure 19. THE SYNTHESIS OF NUCLEOCIDIN.

A group of reagents, the fluoroxyperfluoro-alkanes ($C_nF_{2n-1}OF$), which in effect, generate electrophilic fluoride in addition reactions to appropriately activated olefins has been introduced by Barton et al⁷³. The most frequently used member of the series, fluoroxytrifluoromethane (CF_3OF) adds to activated olefins by two mechanisms⁷⁴ (Figure 20)²⁰. For example, addition of CF_3OF to a glycal (70) yields the ion-pair (71,72) which can collapse to give trifluoromethyl glycoside (73) or it can take second reaction pathway to give the difluoride (74). The addition products (73,74) are exclusively cis. Thus a convenient route for the synthesis of 2-deoxy-2-fluoro-D-glucose⁷⁵ and 2-deoxy-2-fluoro-D-galactose^{76,77} involves the addition of CF_3OF to 3,4,6-tri-O-acetyl-D-glucal and 3,4,6-tri-O-acetyl-D-galactal respectively.

Synthetic applications of this reagent for the synthesis of 2-deoxy-fluoro-disaccharides have been limited because of susceptibility of the disaccharide linkage to the aqueous mineral acid. Nevertheless, the addition reaction of CF_3OF to a disaccharide glycal¹¹ has been studied by Kent and Dimitrijevič. Thus CF_3OF reacts with hexa-O-acetyl-D-lactal (75) to give four fluorinated disaccharides (Figure 21).

The preponderance of the adduct (76) and (77) having β -D-manno configuration was interpreted as due to steric hindrance of the non-reducing (galactoside, B) ring of the disaccharide glycal to the approach of the reagent from the α -D-glucose (ring A) side.

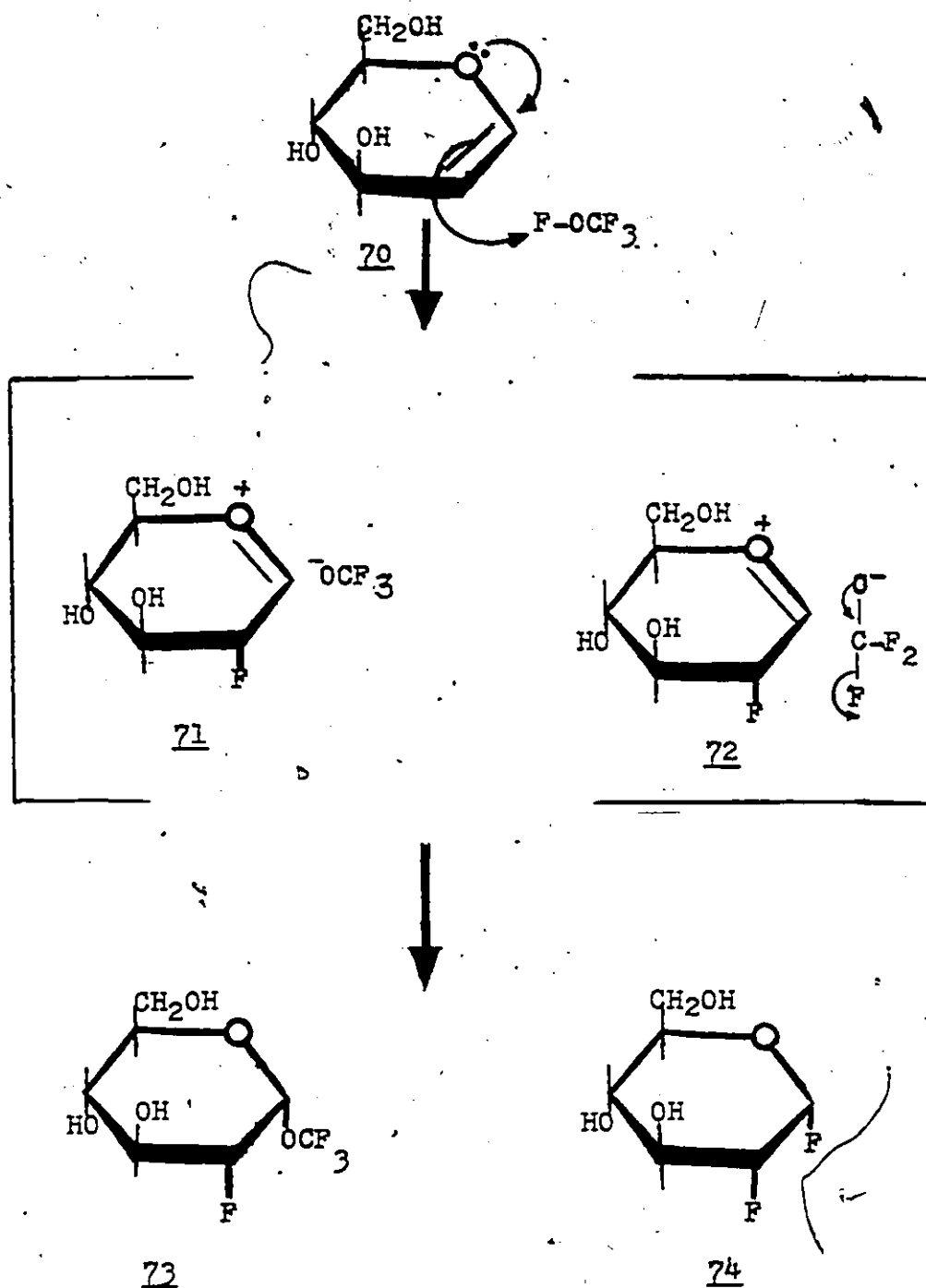
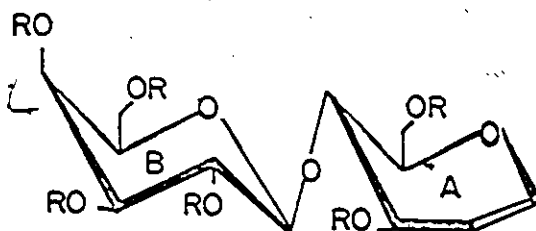
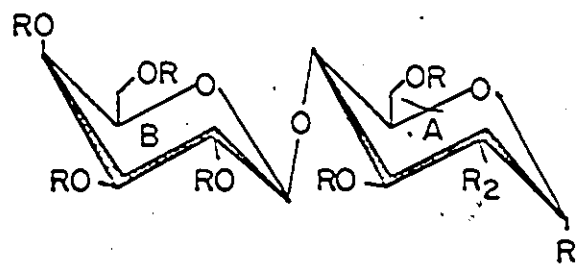
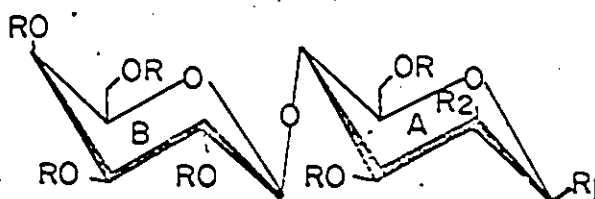


Figure 20. A MECHANISM OF ADDITION OF FLUOROXYTRIFLUOROMETHANE.



75 R = Ac



76 R = Ac, R₁ = OCF₃

78 R = Ac, R₁ = OCF₃, R₂ = F

R₂ = F

77 R = Ac, R₁ = R₂ = F

79 R = Ac, R₁ = R₂ = F

Figure 21. THE ADDITION OF CF₃OF TO A HEXA-O-ACETYL-D-LACTAL

In the steroid series^{78,79} PbF_4 has been successfully employed in the synthesis of vicinal difluoride from alkenes. With triacetylglucal (61), however this reagent leads to 1,1-difluoro 2,5-anhydro sugar (81). The reaction was postulated⁸⁰ to involve formation and rearrangement of the 1,2-difluoride (80) (Figure 22).

IV Nucleosides that contain fluorine in the carbohydrate moiety

2'-deoxy-2'-fluoro analogs of uridine, 5-fluoro-uridine, ribothymidine⁶⁹ and cytidine⁸¹ have been prepared by the action of HF on 2,2'-anhydro nucleoside in dioxane. Nucleocidine (1) has been prepared by glycal addition reaction with the mixed halogen. Clearly the above mentioned procedures will limit the type and number of fluoronucleosides that can be prepared. This difficulty was partly overcome by the use of a direct condensation reaction to the fluorosugars with the appropriate base. Three different types of condensation reactions have been successfully employed for the synthesis of fluoronucleosides.

IV-A Chloromercuri condensations⁶²

Refluxing 2,5 di-O-benzoyl-3-deoxy-3-fluoro- α , β -D-xylofuranosyl bromide (82) with chloromercuri-6-benzamido purine in xylene, followed by debenzoylation gave the β -glycosidic nucleoside (83). Since the 2-O-benzoyl substituent could participate during the condensation reaction, only the

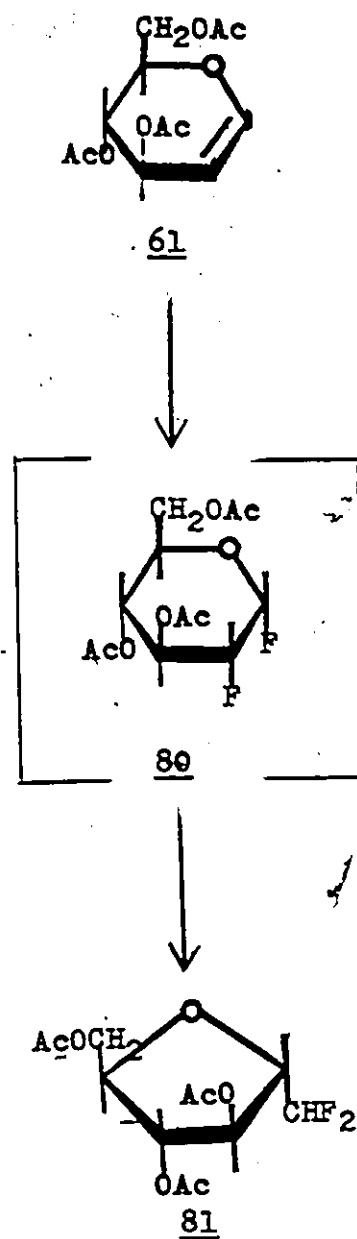


Figure 22. THE ADDITION OF PbF_4 TO A GLYCAL.

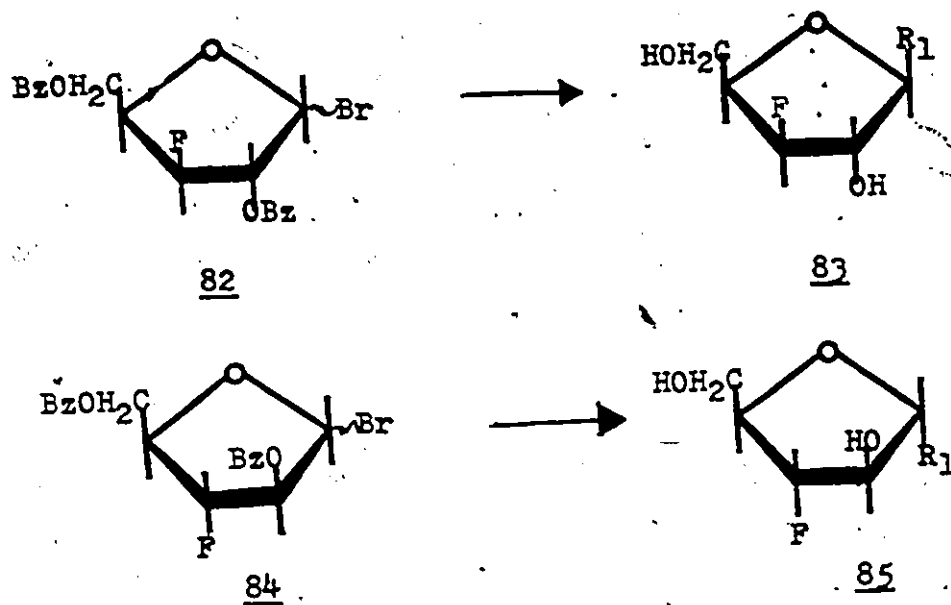


Figure 23. THE CHLOROMERCURI CONDENSATION.

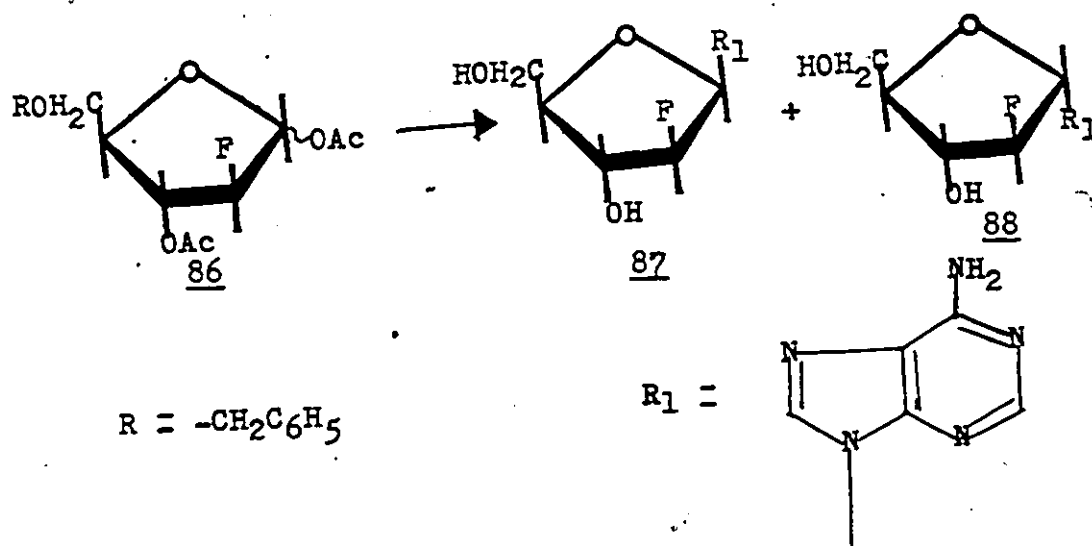


Figure 24. THE FUSION REACTION.

β -anomeric nucleoside was formed. In the arabinose series, treatment of 2,5-di-O-benzoyl-3-deoxy-3-fluoro- α,β -D-arabinofuranosyl bromide (84) under similar conditions gave α -glycosidic nucleoside (85) as a major product (Figure 23).

IV-B Fusion reaction⁶¹

Only one fusion reaction has been reported⁶¹ in the fluorinated nucleosides. The condensation of (86) with 2,6-dichloropurine in the presence of p-toluene sulphonic acid gave the mixture of α - and β -glycosides (87,88) (Figure 24).

IV-C Silyl procedure^{82,83}

Fox *et al* used the 'silyl procedure' to prepare different cytosine nucleosides^{82,83}. The treatment of (89) and (91) (Figure 25) with bis (trimethylsilyl) cytosine followed by debenzoylation gave the corresponding β -glycosidic nucleosides (90) and (92). The β -configuration in (90) was expected on the basis that the benzoyl group at C₂ of bromosugar (89) could participate during the condensation reaction. The most plausible explanation for predominance of the β -glycosidic nucleoside product (92), in view of the absence of a participating group at C₂ of the sugar, is that the bromosugar (91) exists largely as the α -anomer, and undergoes direct S_N2 attack by N₁ of the base to give a preponderance of the β -glycosidic nucleoside. Treatment of (93) under similar conditions gave a mixture of α - and β -glycosidic nucleosides (94) and (95).

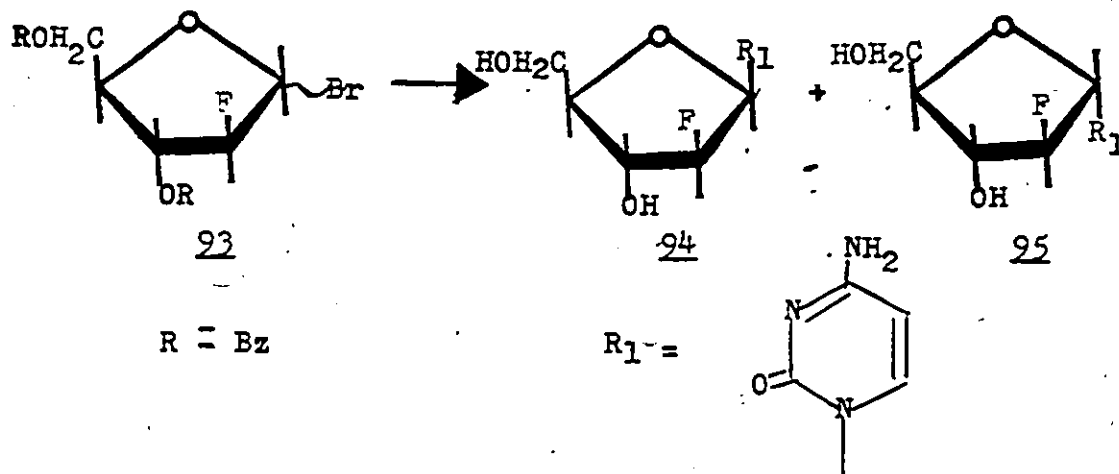
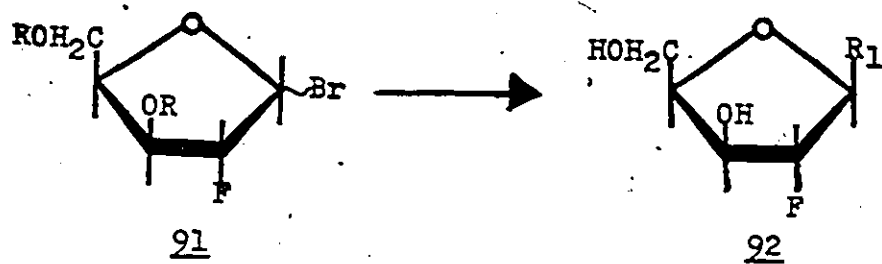
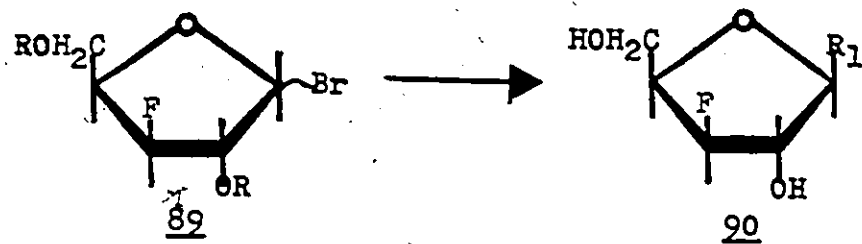


Figure 25. THE 'SILYL' PROCEDURE.

Objectives

The objectives of the present work can now be stated.

1. To develop an alternative synthesis to that reported⁶⁶ for 4-deoxy-4-fluoro-D-glucose which is amenable to the introduction of uniformly labelled ^{14}C or stereospecifically labelled ^3H .
2. To use 4-deoxy-4-fluoro-D-glucose as a probe for the binding specificity of D-glucose to the transport carrier system in the human erythrocyte¹⁶⁹.
3. To synthesise $^3\text{H}-(\text{C}_3)$ -3-deoxy-3-fluoro-D-glucose $^3\text{H}-(\text{C}_1)$ -3-deoxy-3-fluoro-D-glucitol. The former compound is required for a study of glucose transport in *Ps. fluorescens*¹⁷³ and rat brain synaptosomes¹⁷⁴ and the latter compound for elucidating the biochemical mode of toxic action of 3-deoxy-3-fluoro-D-glucose in *locusta migratoria*⁸.

CHAPTER 11

RESULTS AND DISCUSSION

The Fischer Kiliani Cyanohydrin reaction of 3-deoxy-3-fluoro-D-arabinose.

The base or acid catalyzed reaction of hydrocyanic acid with aldehydes and ketones gives two isomeric 2-hydroxynitriles, hydrolysis of which affords 2-hydroxyacids. Originally this reaction was applied by H. Kilian⁸⁴ to D-fructose, D-glucose, L-arabinose and D-galactose. The intermediary cyanohydrins are not usually isolated, but hydrolyzed into the corresponding aldonic acids. The formation of a new asymmetric centre gives rise to two 2-epimers, usually in unequal amounts. The proportions vary according to the stereochemistry of the monosaccharide used and the reaction conditions. Two resultant acids can usually be separated by fractional crystallization of the derived metal salts, lactones, phenylhydrazides, or amides.

When aqueous hydrocyanic acid containing a little ammonia is used, some stereoselectivity may be observed in the reaction with the aldoses, inasmuch as the 2,4-threo product preponderates over the 2,4-erythro isomer⁸⁵. This selectivity can be explained by the use of Cram's rule^{17,18} in which the nucleophile (CN^-) approaches the carbonyl group from the side of the smallest group (Figure 26). However, the alteration of the reaction conditions can reverse the stereoselectivity. Militzer⁸⁶ found that for all simple sugars the optimum reaction occurs at pH 9 to 9.1, even with the sto-

ichiometric amount of cyanide.

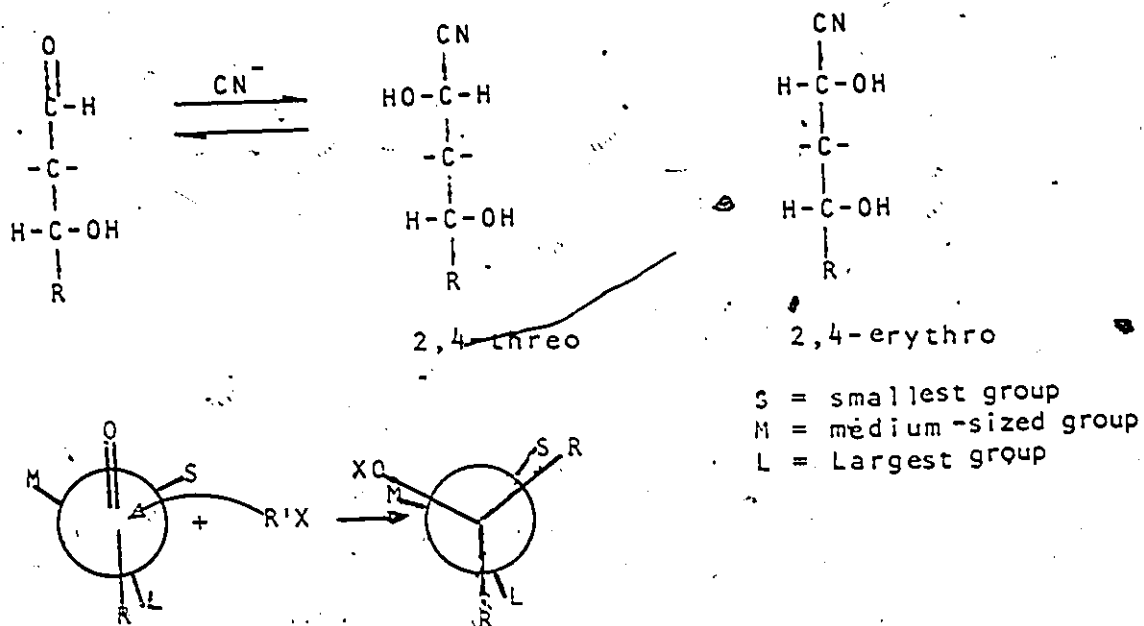


Figure 26: CYANOHYDRIN REACTION

The extension of this reaction to the ascent of the aldose series was made possible by Emil Fischer's observation that aldonolactones, derived from aldonic acids, are reduced to aldoses by sodium amalgam under mildly acidic (pH 3.0 to 3.5) conditions⁸⁷. This sequence of reactions of converting aldehyde into one higher carbon aldehyde is known as Fischer-Kiliani reaction. In view of the wide applications of this reaction to the synthesis of rare sugars from the readily available sugars^{84,88}, it was inevitable that this method would be attempted sooner or later for the syntheses of fluorocarbohydrates. Since one of the aims of the present programme is to investigate a method for the preparation of (14_C)-4-deoxy-4-fluoro-D-glucose, the cyanohydrin reaction of 3-

deoxy-3-fluoro-D-arabinose (3FA) was studied.

With this aim in view, 3FA was synthesized in nine steps from the readily available D-xylose by a route described by Wright and Taylor⁶³ (Figure 27).

The separation of the two anomers was carried out by vacuum-fractionation of the methyl 3,5-O-isopropylidene- α , β -D-xylofuranoside (99), the difference in the volatility between the two being due to the existence of intramolecular hydrogen bonding in the α -anomer between the glycosidic oxygen and the C₂-hydroxyl group. The α -anomer was used for the next reaction sequence. Epoxide opening of the compound (103) with KHF₂ resulted in the desired product. However, the present author observed that the KHF₂ reaction with (103) goes almost to completion; thus there was not a significant amount of unreacted starting material left, contrary to the published report⁶³. The product was isolated by p.l.c. as reported. After removing the blocking groups, 3FA was isolated as colourless crystalline compound and characterized by m.p., ¹⁹F n.m.r. and t.l.c.

A study of the cyanohydrin reaction was now undertaken. Reaction conditions were similar to those described by Isbell et al⁸⁸. Accordingly, 3FA was treated with sodium cyanide in alkaline aqueous solution at room temperature and reaction was monitored by t.l.c. (solvent A). Reaction ap-

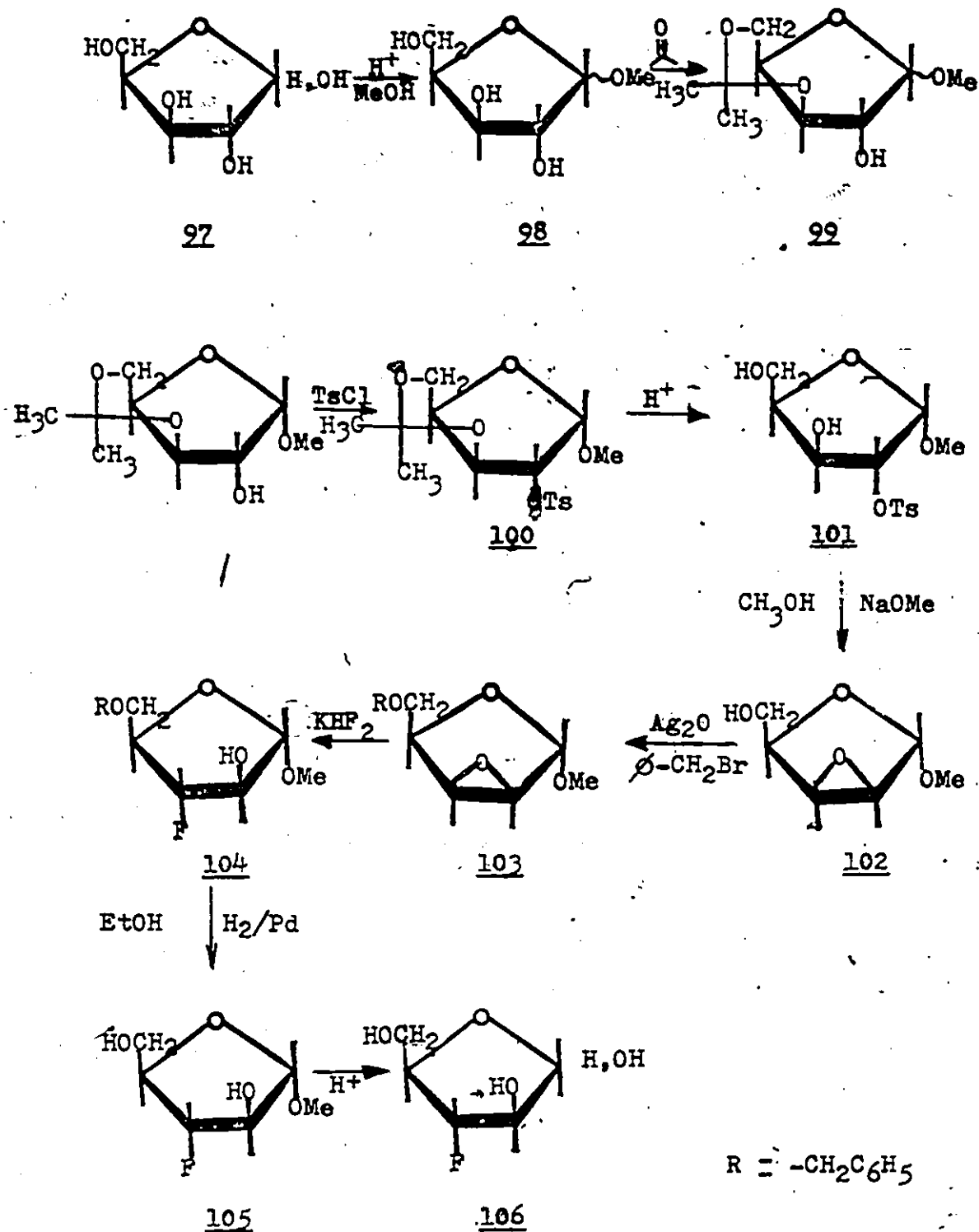


Figure 27. THE SYNTHESIS OF 3-DEOXY-3-FLUORO-D-ARABINOSE.

peared to be complete after three days. The two epimeric cyanohydrins (107) were not isolated but hydrolysed into the acids by heating over steam bath. The alkaline hydrolysis of fluorocyanohydrins (107) was found to be slow compared to the corresponding non-fluorinated analogues and therefore longer reaction time was necessary.

Since the barium salt of D-gluconic acid is insoluble in aqueous methanol (50%) and the barium salt of D-mannonic acid is soluble, two epimeric acids were separated as their barium salt by Isbell *et al*⁸⁸. The same technique was used to separate the two epimeric acids which resulted from the fluorocyanohydrins (107). Both fractions, (methanol soluble and methanol insoluble), however, in this case, were found to be chromatographically similar (solvent S, solvent O). The crude syrup did not crystallise. The i.r. spectrum of the syrup (methanol soluble fraction and methanol insoluble fraction) showed a strong band at 1630 cm^{-1} indicating the presence of C=C and ^{19}F n.m.r. of the syrup showed no signal for fluorine. Therefore, it was concluded that the alkaline hydrolysis of fluorocyanohydrin (101) may have lost fluorine to yield an unsaturated product. Since the objectives of this project were synthetic rather than mechanistic, the unsaturated sugar was not characterized rigorously and no attempt was made to study the re-

action mechanism of alkaline hydrolysis of the fluorocyanohydrines (107). Sodium borohydride reduction of (108) was found to be incomplete. A non-reducing unsaturated product was isolated which was tentatively assigned structure (109) (figure 28), along with some unreacted starting material.

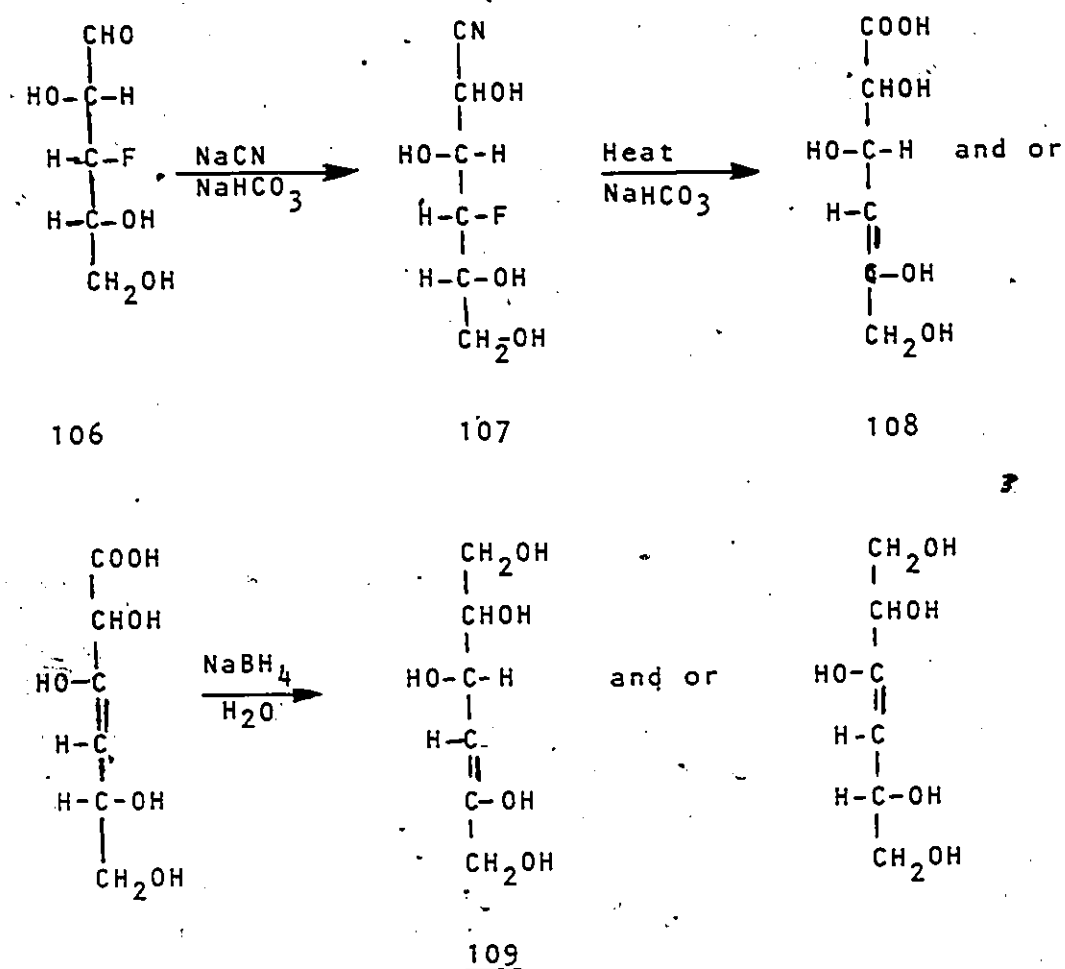


Figure 28 FISCHER-KILIANI REACTION OF 3FA

Sowden-Fischer reaction with 3FA

The carbon chain of the aldose may be extended by a base catalyzed aldol type of addition reaction with nitromethane. The electron-withdrawing nitro group of nitromethane facilitates the formation of the resonance stabilized carbanion ($^-\text{CH}_2\text{NO}_2$), which attacks the electron deficient carbon atom of the carbonyl group to give a 1-deoxy-1-nitro-alditol. Subsequent decomposition of the aci form of the nitro-alcohol in strongly acidic solution, known as the Nef reaction, gives the hydroxyaldehyde (Figure 29).

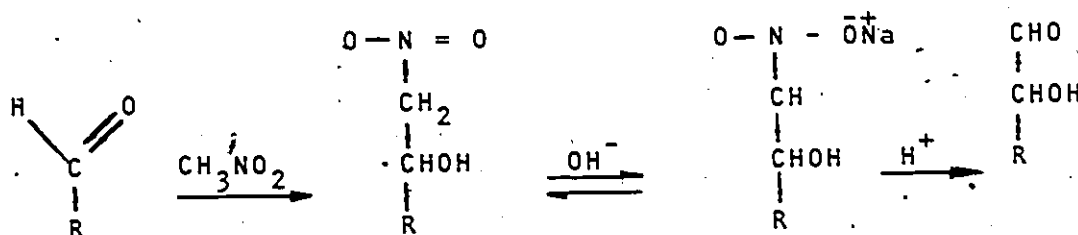


Figure 29. THE SOWDEN-FISCHER REACTION

The nitromethane reaction can be effected conveniently with the unsubstituted aldose. Since the reaction is reversible, a large excess of nitromethane is used with 1 to 2 molar equivalents of alkali⁹⁰. Best yields are obtained by using a solvent in which the starting material is soluble but from which the resulting sodium salt of

the aci-nitroalcohol precipitates. In most cases methanol, sodium methoxide is used, although the use of dimethyl sulfoxide as a solvent has been reported to give improved yields⁹².

As in the cyanohydrin synthesis, a new asymmetric centre is created at C₂, giving rise to two epimers, which generally can be separated by fractional crystallization of the 1-deoxy-1-nitro-alditols. Usually some stereoselectivity is observed⁹³, so that one epimer preponderates. Like the cyanohydrin reaction, the major product is that isomer in which the 2,4-hydroxyl groups have a threo relationship⁸⁵.

This reaction has been widely used in the preparation of higher carbon sugars⁹⁰, deoxysugars⁹⁰, ketoses⁸⁴, and ¹⁴C-sugars⁸⁴. Since the cyanohydrin reaction failed to give the desired product, the Sowden-Fischer reaction with 3FA was studied. Reaction conditions were similar to those described by Sowden *et al*⁹¹. Accordingly, 3FA was allowed to react with nitromethane for 24 hours at room temperature and the product was crystallized from absolute alcohol. Fractional crystallization of the product from ethyl alcohol gave two chromatographically identical (solvent B, solvent P) crops of the crystalline product which had identical m.p. The product was not reducing to aniline phthalate spray and showed strong absorption at 1540 cm⁻¹ in the i.r. spectrum, indicating the presence of the nitro group. The elemental analysis corresponded to the expected 4-deoxy-4-fluoro-1-deoxy-1-nitrohexitol (4FNH) (110).

The 4FNH was subjected to the Nef reaction, using Amberlite

IR 120 (H^+) as a acid catalyst. The product was identified by comparative t.l.c. as a mixture of 3FA and 4FNH. The i.r. spectrum (KBr) of the product was identical to the i.r. spectrum of the mixture of 3FA and 4FNH (1:1 w/w). This observation was further confirmed by the field desorption mass spectrum of the product which contained the following peaks expressed as m/e (% abundance using m/e 31 as the base peak): 214 (6), 152(4), 123(40), 112(4), 90(3), 89(3), 62(6), 61(90), 46(37), 31(100). The peaks at m/e 214 and m/e 152 can be derived as a molecular peak for 4FNH ($M^+ + 1$) and 3FA (M^+). Other peaks can derived from 4FNH and 3FA or both by loss of $-NO_2$ (46), $-CH_2OH$ (31), $-CH_2NO_2$ (60), $-CHOH-CH_2-NO_2$ (90, 123) etc.

Even though the formation of 3FA by weak acid hydrolysis of 4FNH was rather surprising, regeneration of 4FNH was expected, since a similar result has been reported by other workers⁹⁰. Therefore the hydrolysis of 4FNH by strong acid (H_2SO_4) was undertaken. The syrupy residue obtained by hydrolysis of 4FNH with sulfuric acid did not crystallize. Comparative cellulose t.l.c. of the product (plate 1 page 151) indicated it to be a mixture of at least two reducing sugars. The faster moving component had an R_F value higher than 3FA and 4-deoxy-4-fluoro-D-glucose (4FG), and was therefore, assigned as 4-deoxy-4-fluoro-D-mannose. The reference samples used for the comparison were 3FA and 4FG both with R_F values the same as the slower moving component of the reaction product. The colour of the slower moving component with aniline-phthalate spray was identical to that of 3FA (dark brown) and was different from that of 4FG (faint yellow). On plate 2 (page 151) both reaction products separated well

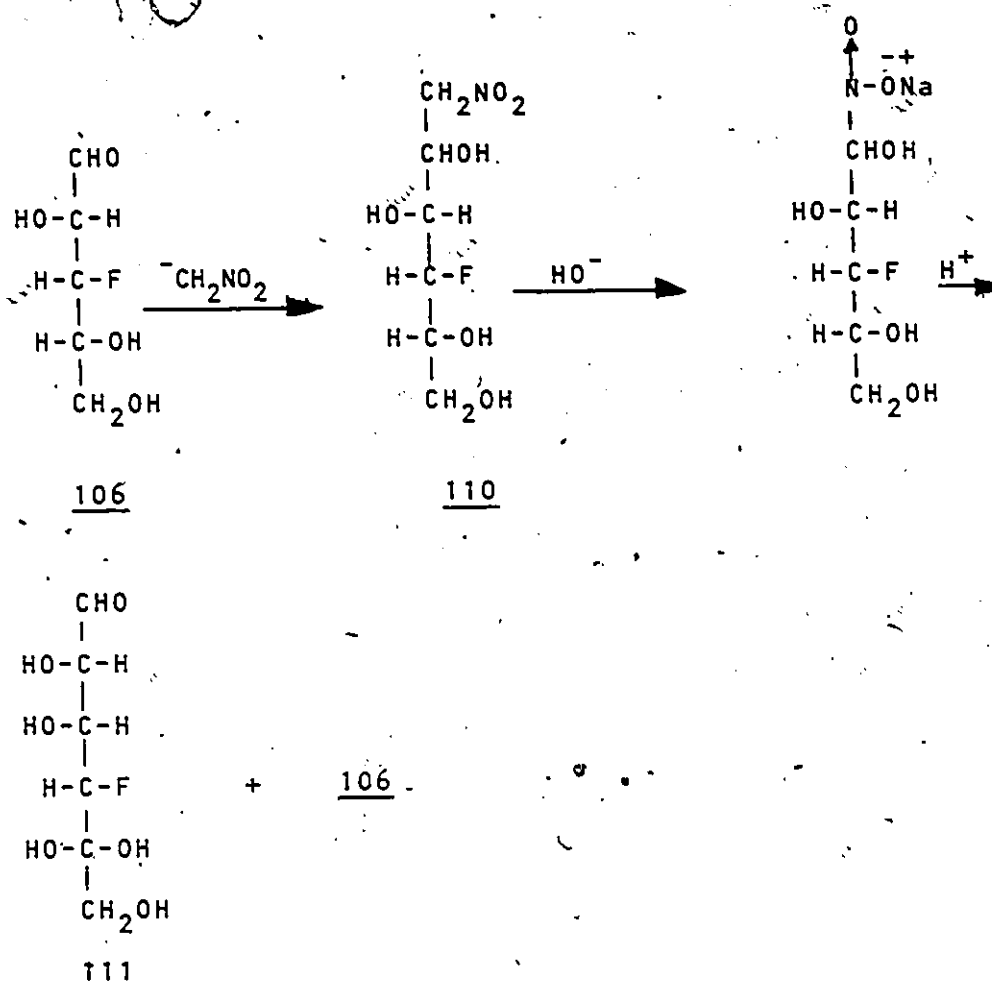


Figure 30. SOWDEN-FISCHER REACTION OF 3-DEOXY-3-FLUORO-D-ARABINOSE.

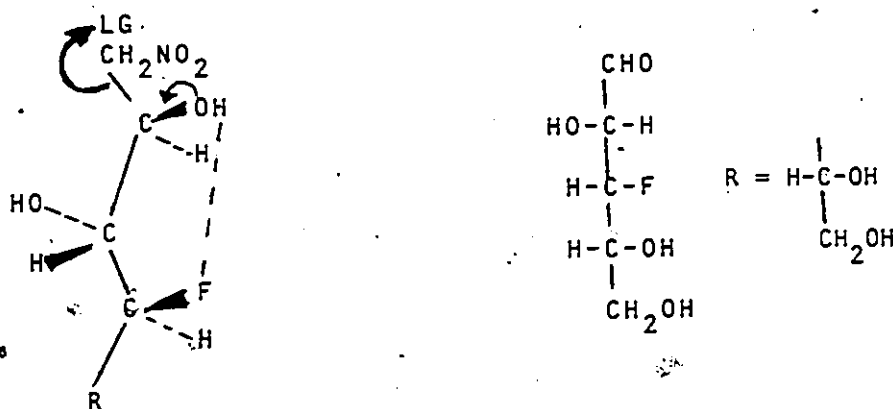


Figure 31. A MECHANISM FOR THE FORMATION OF 3-DEOXY-3-FLUORO-D-ARABINOSE FROM 4-DEOXY-4-FLUORO-1-DEOXY-1-NITRO-HEXITOL.

and the faster moving component had the same R_F value and colouration with aniline phthalate spray as that of 3FA.

Therefore it was concluded that the probable reaction products of acid hydrolysis (H_2SO_4) of 4FNH are 3FA and 4-deoxy-4-fluoro-D-mannose (111). The reaction sequence of Sowden-Fischer reaction with 3FA is shown in Figure 30. In the zigzag conformation, the 4-fluoro group and C_2 -OH group come into close proximity, particularly in the gluco conformation, which may explain the formation of 3FA and absence of 4-deoxy-4-fluoro-D-glucose as a reaction product. A possible reaction mechanism is shown in Figure 31.

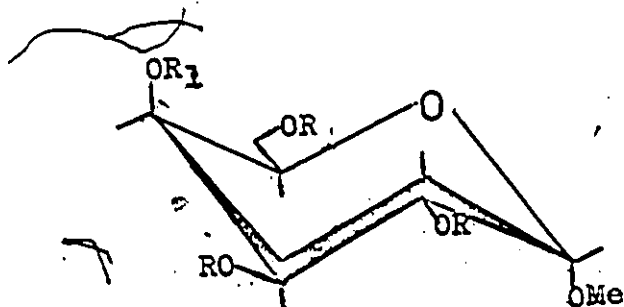
The synthesis of 4-deoxy-4-fluoro-D-glucose.

Two main problems were encountered in dealing with the cyanohydrin and Sowden-Fischer reactions of 3FA.

- (1) The behaviour of the 3FA and 4FNH in strong alkaline conditions is not well understood.
- (2) Starting material (3FA) for this kind of reactions is not readily available and involved multistage synthesis. Since the nucleophilic displacement of a carbohydrate secondary sulphonate has been done successfully, in good yield, using a fluoride salt⁵⁴, a study in this direction was undertaken.

Taking into consideration the requirements for the displacement reaction of the C_4 sulphonyloxy group³⁹ (see in-

production) and keeping in mind the objective of the project, the ideal compound to study for nucleophilic substitution reaction with a fluoride salt will be the galactose derivative (117). The benzyl groups at C₂, C₃ and C₆ were preferred to benzoyl groups for the greater stability of the former in an acidic or basic medium and ease of debenzylation to give a free glycoside. Also, benzoyl groups, like acetyl groups, complicate the isolation of the product by a neighbouring group participation²⁹. The α -isomer was chosen because its ease of preparation.



| | |
|-----|----------------------|
| 113 | $R_1 = R = -OH$ |
| 117 | $R_1 = -CH_2-C_6H_5$ |

Since in a free glycoside (113), all free -OH groups are equatorial except C₄-OH, which is axial, some selectivity benzoylation has been observed¹⁰⁵. Therefore benzoylation of (113) was studied with benzyl bromide and silver oxide in dimethyl formamide (DMF). The ratio of benzyl bromide to (113) was varied from 3:1 to 9:1 (on molar basis) to establish optimum reaction conditions which gave a product having 15 aromatic protons per -OMe group. The optimum

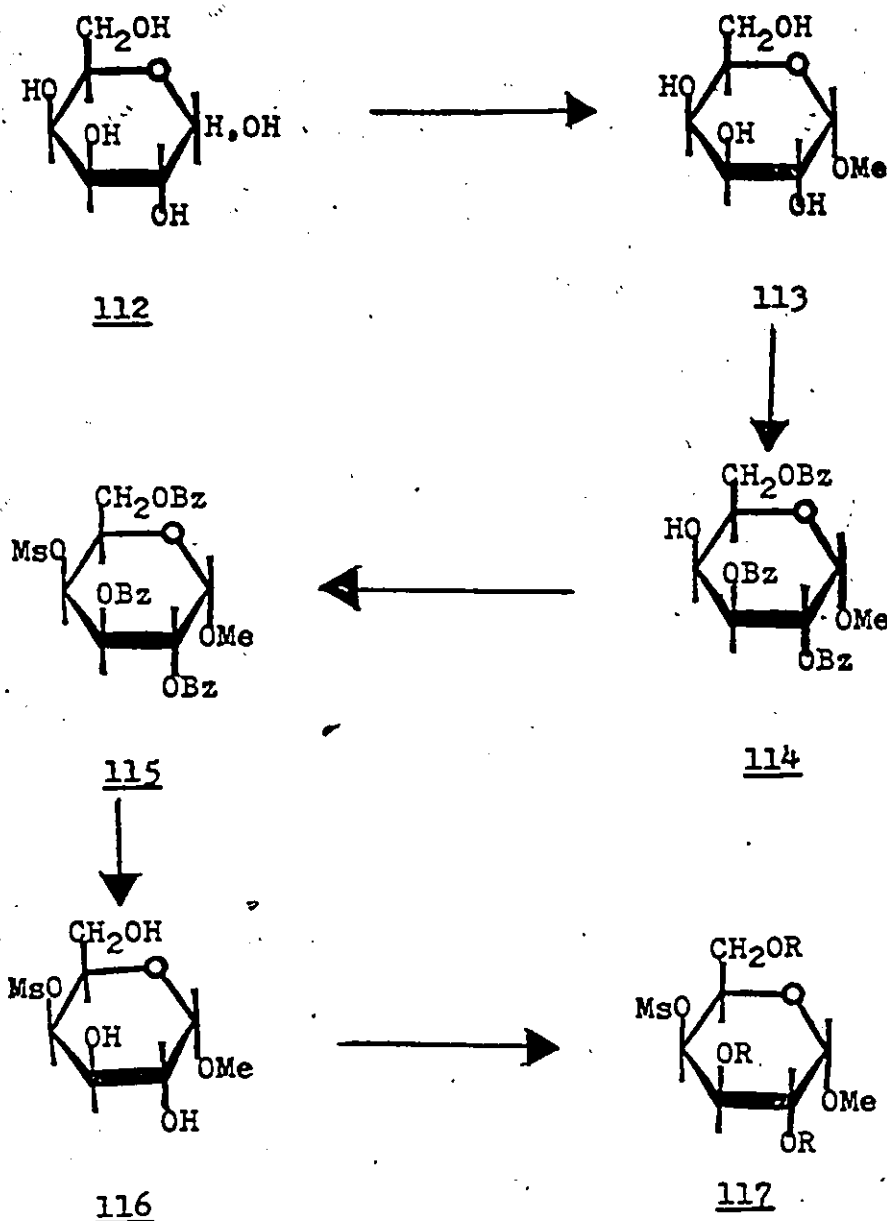
was found to be the 9:1 molar ratio. The product was purified from non-sugar impurities by p.l.c. (solvent J). However it showed very complex (more than 7 components) on t.l.c. (solvent E). The product had expected i.r. absorption at 3600 cm^{-1} indicating the presence of a hydroxyl group. The n.m.r. of the product was rather complex, showing the presence of approximately 15 aromatic protons and 14 aliphatic protons per -OMe group. The temperature of the reaction was varied from room temperature to 40° , but the complexity of the reaction product on t.l.c. (solvent E) remained the same. The above crude product was mesylated with methylsulphonyl chloride in pyridine. This product showed three spots on t.l.c. (solvent G). All three components were isolated by p.l.c. (solvent G) and were about equal yield. They had similar i.r. spectra (absence of -OH group) and showed one mesyl group per OMe group (by n.m.r.). However, the elemental analysis (C,H,S) of the products was unsatisfactory.

Preliminary studies of selective benzylation using other reagents (benzyl chloride and NaH) were far from encouraging. There was almost no reaction below 50° and at a temperature above 85° only fully benzylated product was isolated. A recent report¹⁰⁶ on selective benzylation of methyl- α -D-galactopyranoside derivatives, showed that reactivity of -OH groups in (113) are in the following order for the benzylation reaction: $C_6\text{-OH} > C_4\text{-OH} = C_2\text{-OH} > C_3\text{-OH}$. Therefore further

work in this direction was abandoned.

Another way of making compound (117) is to benzylate methyl 4-O-methylsulphonyl- α -D-galactopyranoside¹⁰⁷ (116). Therefore compound (116) was prepared from readily available D-galactose by a sequence, D-galactose \rightarrow methyl- α -D-galactopyranoside¹⁰⁸ \rightarrow methyl 2,3,6-tri-O-benzoyl- α -D-galactopyranoside¹⁰⁵ \rightarrow methyl 2,3,6-tri-O-benzoyl-4-O-methylsulphonyl- α -D-galactopyranoside¹⁰⁷ \rightarrow methyl-4-O-methylsulphonyl- α -D-galactopyranoside¹⁰⁷ (111). Since a trans position of a leaving group is required for epoxide formation in which oxygen anion at one carbon atom attacks the adjacent carbon atom from the side opposite to that from which the anionic 'leaving group' departs, debenzoylation of (115) did not produce the epoxide which in turn proved that the mesyl group is indeed at C₄ position in compound (116). The yield of the debenzoylation reaction was improved to 75% compared to the reported¹⁰⁷ 58% by slightly modifying the reaction conditions, (a longer reaction time and the use of ion exchange resin for neutralization of reaction mixture).

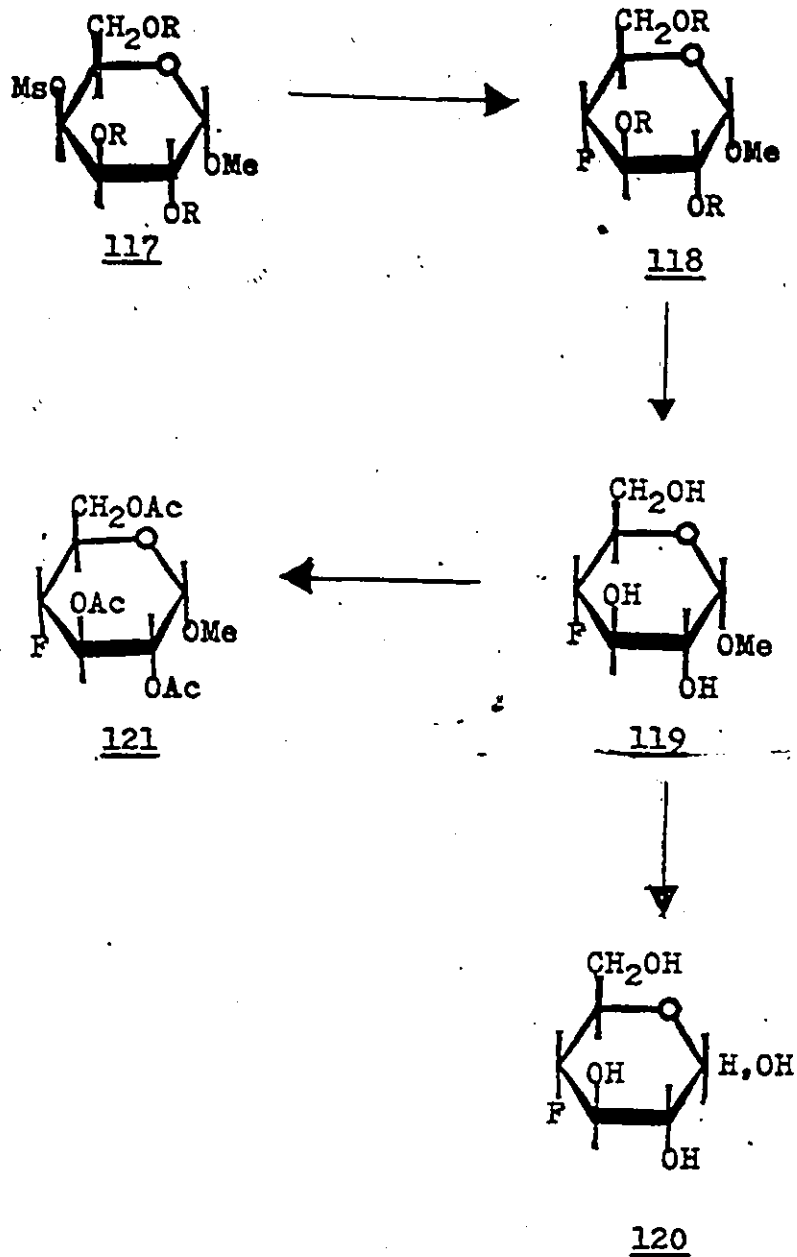
The benzylation of (116) using about two times excess of benzyl bromide in the presence of silver oxide in DMF at room temperature proceeded smoothly. The reaction was monitored by t.l.c. (solvent F) and found to be complete in three days. The product was purified from non-sugar impurities by column chromatography over alumina. Washing the column with pet. ether: ether (100:20) removed the dibenzyl ether (identi-



$\text{R} = -\text{CH}_2\text{C}_6\text{H}_5$

Figure 32. THE SYNTHESIS OF 4-DEOXY-4-FLUORO-D-GLUCOSE.

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fied by n.m.r.) as an impurity. Elution with ether gave the desired product as a colourless mobile syrup, leaving behind unidentified coloured impurities on the column. The i.r. spectrum of the product had three bands in $710-790\text{ cm}^{-1}$ region characteristic of $-\text{C}_6\text{H}_5$ grouping. The absence of absorption around $3200-3500\text{ cm}^{-1}$ indicated substitution of hydroxyl groups. The weak bands in the region $1600-2000\text{ cm}^{-1}$ indicated mono substituted benzenes. The n.m.r. spectrum was consistent with the assigned structure as methyl 2,3,6-tri-O-benzyl-4-O-methylsulphonyl- α -D-galactopyranoside. The anomeric proton ($\text{C}_1\text{-H}$) showed as a doublet ($J=2.5$), but other ring protons ($\text{C}_2\text{-H}$, $\text{C}_3\text{-H}$, $\text{C}_4\text{-H}$, $\text{C}_5\text{-H}$, and $\text{C}_6\text{-H}_2$) showed as a complex multiplet ($\delta = 4.1-3.5$) and the interpretation was difficult.

Methyl 2,3,6-tri-O-benzyl-4-O-methylsulphonyl- α -D-galactopyranoside (117) on treatment with tetrabutylammonium fluoride (Bu_4NF) in acetonitrile reacted by refluxing. The reaction was completed in three days. The products were obtained by pouring the reaction mixture into water and followed by extraction with ether. The ether extract showed two main spots (solvent G). The product was (R_F 0.63, solvent G) isolated by column chromatography over silica gel by eluting with ether-pet ether (70:30, v/v). The elemental analysis (C,H,F) corresponded to the expected methyl 2,3,6-tri-O-benzyl-4-deoxy-4-fluoro- α -D-glucopyranoside. The i.r. spectrum of the product had a strong absorption at 1050 cm^{-1} indicating the presence of C-F bond and no absorption around $3200-3500$

cm^{-1} region indicating substitution of hydroxyl groups. With the anticipated Walden inversion at C_4 the n.m.r. signal for $\text{C}_4\text{-H}$ was expected to be a widely spaced triplet (or a quartet), however the ring protons of (118), showed as a very complex multiplet and were superimposed over each other. Therefore $\text{C}_4\text{-H}$ could not be distinguished from the rest of the ring protons. A long range coupling between F and anomeric proton ($\text{C}_1\text{-H}$) has been observed by other workers^{47,48}. Therefore a triplet (or a quartet) for anomeric proton was expected for compound (118). However it was shifted slightly upfield ($\delta = 5$) and was superimposed over the benzylic ($\text{CH}_2\text{-C}_6\text{H}_5$) protons. The second component (R_F 0.35, solvent G) of the reaction mixture was found to be very minor ($\sim 1\%$) and was therefore not rigorously identified. Reexamination of the product, however, (R_F 0.35, solvent G) on t.l.c. (solvent H) revealed that it was mixture of at least two products, one of them was chromatographically identical to the starting material (117). The i.r. spectrum of the mixture showed absorption at 1180 cm^{-1} (characteristic of $-\text{SO}_3$) and 1630 cm^{-1} (characteristic of $-\text{C}=\text{C}-$) indicating that it is a mixture of starting material (117) and some unsaturated product. Formation of at least one unsaturated product is not surprising, since the sulphonyloxy group in (117) is in trans diaxial relation with the C_3 and C_5 protons. Similar observations have been reported by other workers⁴⁷.

The benzyl protecting groups were smoothly removed

from the fluoride (118) by hydrogenolysis over palladised charcoal, to give 4-deoxy-4-fluoro- α -D-glucopyranoside (119). Some side products were observed in the hydrogenolysis and the glycoside (119) was recovered by column chromatography over silica gel. The glycoside had the expected elemental analysis (C,H,F). The i.r. spectrum of the product had an absorption at 3380, 3460 cm^{-1} region indicating presence of hydroxyl groups.

The glycoside was further characterized as its triacetate (121) by treating with acetic anhydride in pyridine. The n.m.r. and mass spectral data for the triacetate (121) of (119) confirmed the 4-deoxy-4-fluoro-D-gluco structure. The n.m.r. spectra of (121) in C_6D_6 showed $\text{C}_1\text{-H}$ (anomeric proton) as a triplet due to the long range coupling between fluorine and $\text{C}_1\text{-H}$, and $\text{C}_1\text{-H}$ and $\text{C}_2\text{-H}$. Three pairs of doublets were observed for $\text{C}_4\text{-H}$ with $J_{4,3} = J_{4,5} = 9.5 \text{ Hz}$ and $J_{4,F} = 3.75 \text{ Hz}$. The large values for $J_{4,3}$ and $J_{4,5}$ suggest glucose configuration, since $\text{C}_4\text{-H}$, $\text{C}_3\text{-H}$ and $\text{C}_5\text{-H}$ will be all trans diaxial.

The fragmentation pattern of (121)⁺ under electron impact would be expected to resemble that of the other 4-fluorohexopyranoses^{50,51,111}. Thus Figure 33 represents the expected fragmentation and indeed the ions derived from pathways A,B,C,D,E and F all were seen in the mass spectrum of (121). According to the Biemann-Heyns scheme^{109,110} there are four main fragmentation pathways of hexopyranose acetates following electron-impact. Pathway A involves consecutive

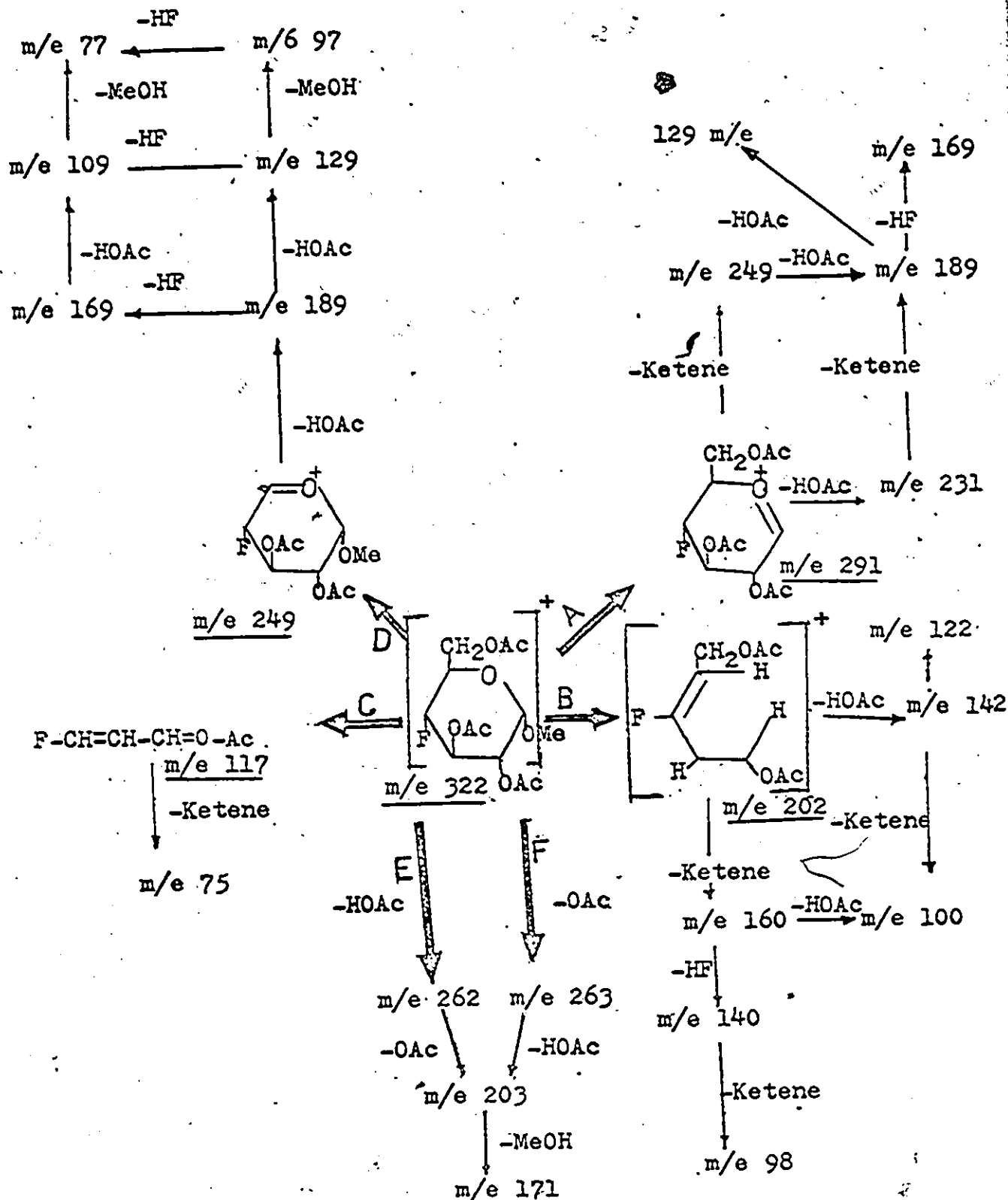


Figure 33. A PROPOSED MASS-SPECTRAL FRAGMENTATION OF METHYL
2,3,6-TRI-O-ACETYL-4-DEOXY-4-FLUORO- α -D-GLUCO
PYRANOSIDE (121).

loss of acetoxy groups (as acetic acid, ketene and structurally related moieties) with retention of the pyranose ring. A second pathway (B), initially involves the loss of C-1 and neighbouring groups (as O-CH-OAc) and also of acetic acid. Subsequent fragmentation occurs by progressive loss of acetic acid and ketene molecules. Pathway C affords a fragment which consists of C-2, C-3 and C-4, and two terminally attached acetoxy groups. A fourth pathway (D) involves the loss of C-6 (as CH₂OAc) followed by loss of acetic acid and ketene molecules. Pathway C would normally (e.g., in a penta-O-acetyl-hexopyranose) provide two prominent peaks in the spectrum. However, the presence of fluorine atom on C-4 makes the cleavage of C-4-C5 and C4-C3 bonds an unfavourable process¹¹¹, resulting in this case in small peaks at m/e 117 and m/e 75, which supports the C4 position of fluorine in compound (116). For two 4-deoxy-4-fluoro-D-glucopyranose derivatives^{50,111}, pathway B produced an intense ions. In the mass spectrum of (121) large peaks appeared at m/e 160, m/e 100 and m/e 202. All these can be expected to derive from pathway B.

Hydrolysis of methyl 4-deoxy-4-fluoro- α -D-glucopyranoside (119) with 2 M sulfuric acid gave the crystalline 4-deoxy-4-fluoro-D-glucopyranose, which was identical (t.l.c., i.r., optical rotation and mixed m.p.) to the authentic⁶⁶ sample*.

The yields in the conversion of methyl- α -D-galactopyrano-

* Kindly supplied Prof. N. F. Taylor.

side (113) into methyl-4-0-methylsulphonyl- α -D-galactopyranoside¹⁰⁷ (116) and various stages of subsequent reaction sequences (116) -72% + (117) -73% + (118) -53% + (119) -74% + 4-deoxy-4-fluoro-D-glucopyranose are uniformly high and the route provides a more ready access to the final product than does the original method⁶⁶. This route, therefore, may be used for the preparation of (¹⁴C)-4-deoxy-4-fluoro- α -D-glucopyranose, since uniformly labelled D-galactose is now available.

The synthesis of 4-deoxy-D-glucose

This compound has been reported^{112,113,114,115} by many workers. The readily accessible methyl 2,3,6-tri-0-benzoyl-4-0-methylsulphonyl- α -D-galactopyranoside (115), however, encouraged a study of its displacement reaction with NaI. Initially the conditions of time and temperature were varied over relatively small range and optimum was found to be refluxing (115) and NaI (1:5 molar ratio) in DMF for one hour. The reaction mixture was poured into the water and extracted with ether. The ether extract on t.l.c. (solvent H) showed the presence of two products and some unreacted starting material (identified by comparative t.l.c., m.p., i.r. and n.m.r.). Two reaction products were separated by column chromatography over silica gel. The faster moving component (R_F 0.51, solvent H) was found to be very minor and was subsequently considered to be methyl 2,3,6-tri-0-benzoyl-4-deoxy

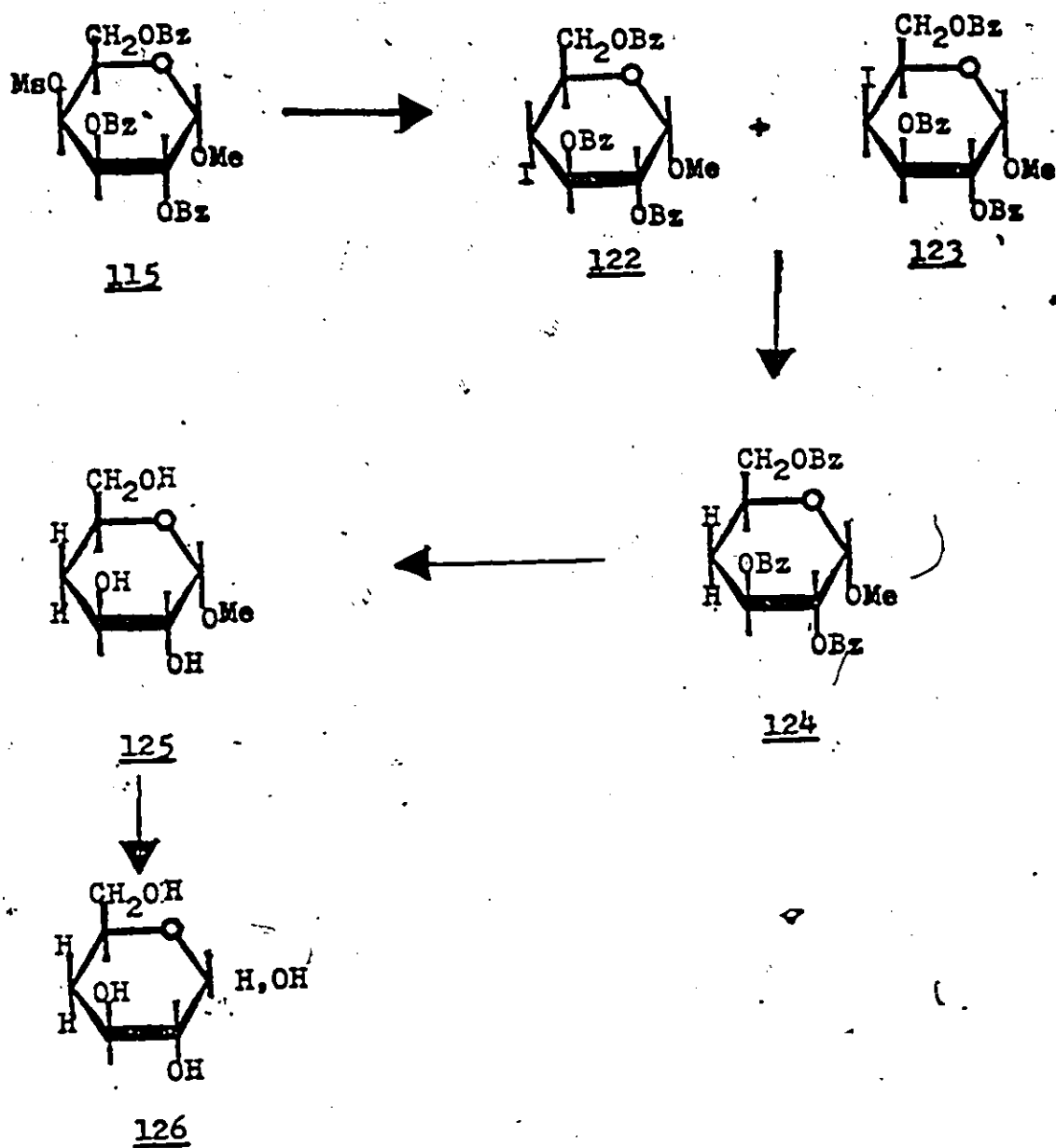


Figure 34. THE SYNTHESIS OF 4-DEOXY-D-GLUCOSE.

-4-iodo- α -D-galactopyranoside (123). The slower moving component (R_F 0.46 solvent H) was found to be the major product and had an elemental analysis (C,H,I) consistent with the empirical formula ($C_{28}H_{25}O_8I$) for methyl 2,3,6-tri-O-benzoyl-4-deoxy-4-iodo- α -D-glucopyranoside (or -galactopyranoside).

The configuration at C_4 was assigned on the basis of its n.m.r. spectrum which showed C_4 -H as an apparent triplet with coupling constant 9.5 Hz. Such a high J was expected for glucose configuration since C_4 -H, C_3 -H and C_5 -H will be all trans diaxial. The glucose configuration was also anticipated with expected Walden inversion.

The compound (123) could not be rigorously identified as it was very minor product (122:123 = 11:1). It was considered to be a galacto derivative because its n.m.r. was different from that of the gluco isomer (122) but contained 15 aromatic and 7 ring protons per-OMe group and had no absorption characteristic of SO_3-CH_3 group in n.m.r. and i.r. spectrum. Final proof for its structure came from the fact that the hydrogenolysis product (H_2/Pd in presence of sodium acetate) of (122) and (123) was identical (chromatography, n.m.r. and m.p.) and had an elemental analysis (C,H) corresponding to the expected methyl 2,3,6-tri-O-benzoyl-4-deoxy- α -D-glucopyranoside. The m.p. of (124) was identical to that reported¹¹². The formation of (123) was not surprising since a similar observation has been reported^{112,116} by other workers. A possible mechanism of formation of (123) is illustra-

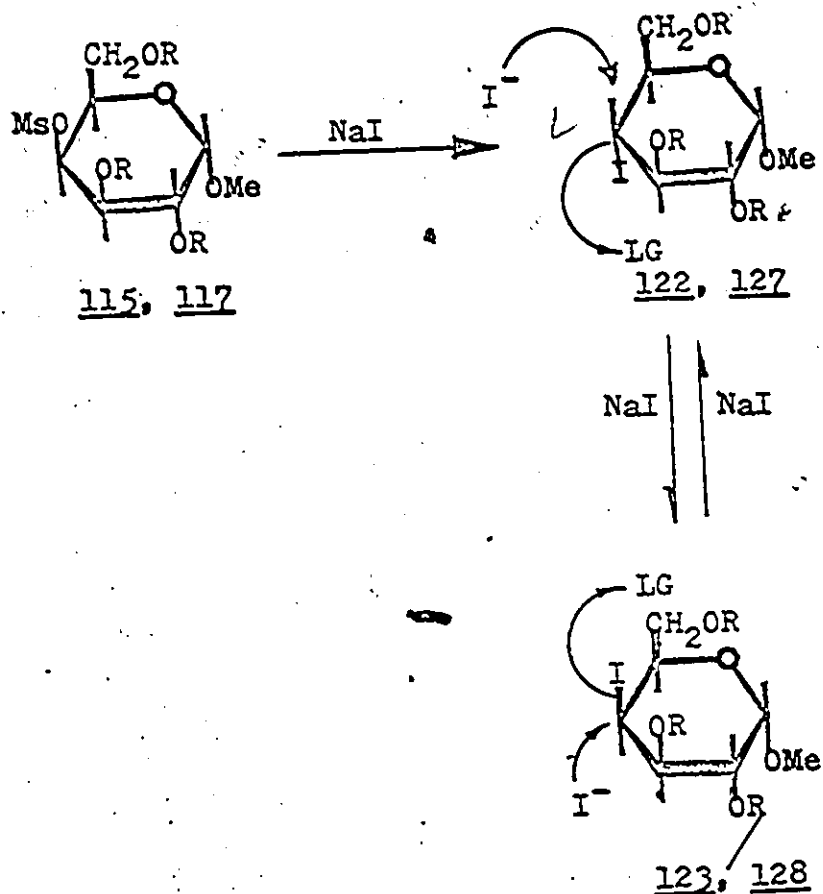
ted in figure 35.

Debenzoylation of (124) gave the free glycoside (125), which by acid hydrolysis (Amberlite IR 120 (H⁺) gave 4-deoxy-D-glucose (126). Physical and chemical characteristics of the glycoside (125) and free sugar (126) were identical to those previously reported^{112.113.114.115}.

Displacement reaction of methyl 2,3,6-tri-O-benzyl-4-O-methylsulphonyl- α -D-galactopyranoside with NaI.

The other compound studied for the displacement reaction with NaI was methyl 2,3,6-tri-O-benzyl-4-O-methylsulphonyl- α -D-galactopyranoside (117). Initially conditions of time and temperature were varied from 1 to 5 hours and 125° to reflux temperature. The optimum reaction conditions were found to be refluxing (117) in DMF with NaI for one hour. The reaction mixture was poured into the water and extracted with ether. The ether extract on t.l.c. showed three products (solvent H) (R_F 0.85, 0.7, 0.6) and some unreacted starting material (identified by comparative t.l.c. and n.m.r.). The above syrupy mixture was submitted to p.l.c. (solvent H); it resolved into three bands, the middle band (R_F 0.7 and 0.6; solvent H) was isolated and had an elemental analysis (C,H,I) corresponding to the empirical formula C₂₈H₃₁O₅I. The n.m.r. of the product showed presence of 15 aromatic protons, 6 benzylic protons (-CH₂-C₆H₅) and 7 ring protons per -OMe group. The anomeric protons showed as singlet (δ = 4.9). The compound was consi-

dered, therefore, to be a mixture of methyl 2,3,6-tri-O-benzyl-4-deoxy-4-iodo- α -D-gluco- and -galactopyranosides (127, 128) (figure 35).



(115), (122), (123) $R = -\text{COC}_6\text{H}_5$

(117), (127), (128) $R = -\text{CH}_2\text{C}_6\text{H}_5$

Figure 35. A PROPOSED MECHANISM FOR THE FORMATION OF GLUCO- AND GALACTO ISOMERS BY THE ACTION OF NaI ON (115) AND (117) IN DMF.

$^3\text{H}-(\text{C}-3)-3\text{-deoxy-3-fluoro-D-glucose.}$

Since 1967, new general methods for the synthesis of fluorosugars have emerged and as a consequence many fluorosugars are now accessible in quantities to permit a thorough evaluation of chemical properties and biological activity (see introduction). As a result of biochemical studies with fluorosugars and related compounds¹¹⁷, it was important to have ^{14}C and/or ^3H -labelled fluoro-glucoses.

Since classical methods of preparing ^{14}C -sugars, such as the Fischer-Kiliani reaction or Sowden-Fischer reaction were unsuccessful when applied to 3-deoxy-3-fluoro-D-arabinose, attention was turned to the preparation of tritium labelled fluoro-glucoses.

From the methods available for the specific introduction of an isotope of hydrogen into a sugar molecule, the most convenient and widely used is the reduction of sugar aldehydes, ketones, lactones, ester groups or halides by a hydride reagent. This method is particularly useful for the preparation of tritiated sugars as ^3H -sodiumborohydride of high specific activity can be obtained commercially. This technique has been extensively used for the preparation of specifically ^3H -labelled sugars and has been reviewed¹¹⁸ by Barnett and Corina. Incorporation of tritium at the secondary carbon atom of the sugar ring depends on the availability of the corresponding ketose derivatives; these may be produced either by bio-

logical or chemical oxidation. Since a ketose derivative (131) has been used in the synthesis of 3-deoxy-3-fluoro-D-glucose⁴³, it was used for the preparation of ^3H -(C-3)-3-deoxy-3-fluoro-D-glucose.

1,2;5,6-di-O-isopropylidene- α -D-ribo-hexofuranos-3-ulose¹¹⁹ (131) was prepared in two steps from D-glucose. Since the ketose derivative (131) is not very stable at room temperature¹²⁰ and difficult to crystallise, it was isolated as its hydrated form (132). The product was chromatographically pure (solvent F) but had a lower melting point than that quoted¹²⁰. The i.r. (KBr) spectrum showed strong absorption at 3400 cm^{-1} and weak absorption at 1780 cm^{-1} indicating it was mixture of ketose (131) and its hydrated form (132). The ratio of (131):(132) was found to be 4:7 by n.m.r. Recrystallization of the above product from acetone gave a colourless needle shaped crystalline compound as the hydrated¹²⁰ form (132).

Since solubility of ^3H -sodium borohydride is very low in absolute ethanol, 80% ethyl alcohol was used as a solvent for the reduction of (131). Decomposition of ^3H -sodium borohydride was minimized by keeping the reaction mixture below 40° . The reaction mixture was poured into water and extracted with ethyl acetate. The product showed two spots on t.l.c. (solvent C), R_F 0.6 (major) and R_F 0.46 (minor) which were consi-

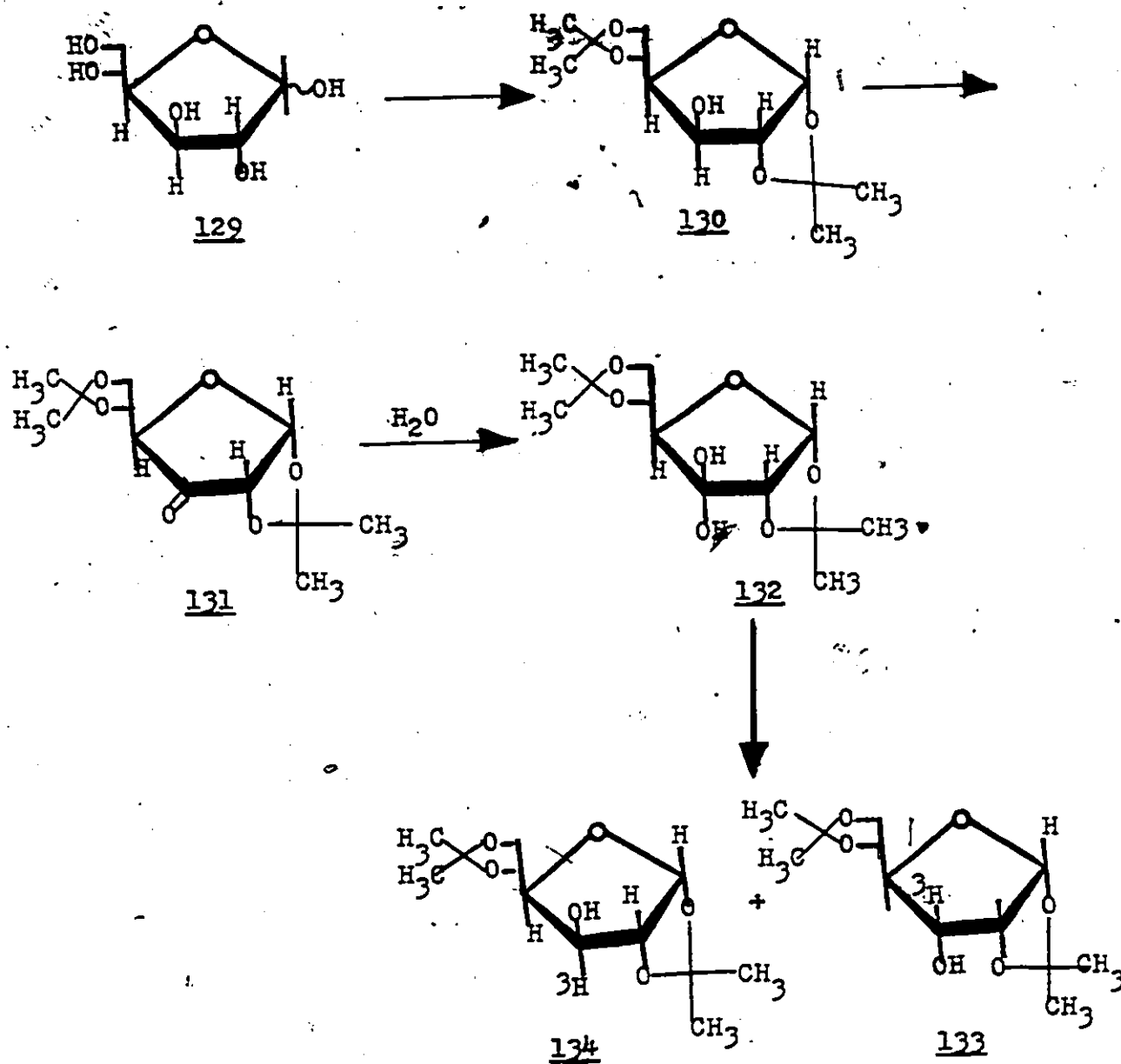
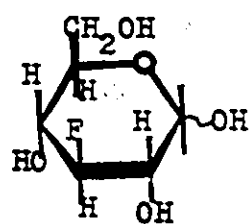
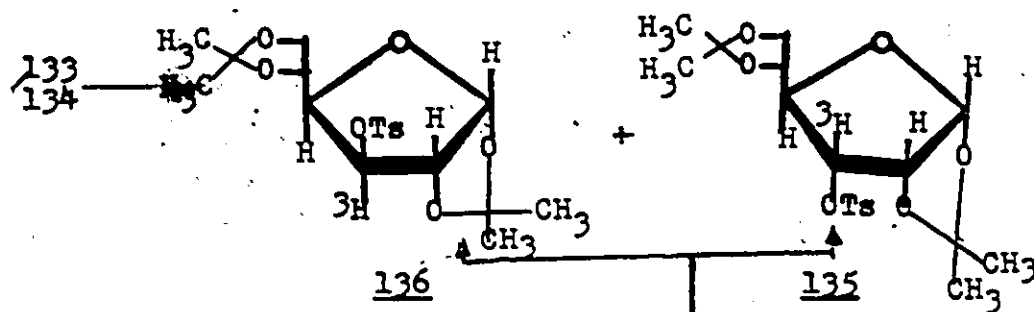
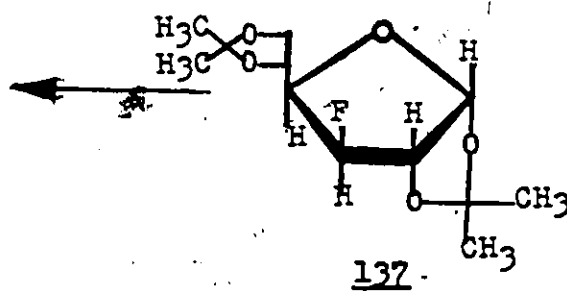


Figure 36. SYNTHESIS OF ³H-(C₂)-3-DEOXY-3-FLUORO-D-GLUCOSE.

Cont. on next page

138137

dered to be the allo (133) and gluco (134) isomers respectively. Even though sodium borohydride reduction of (131) has been reported¹²⁰ to give stereospecifically the allose isomer, the formation of a minor amount of the glucose derivative (134) can not be excluded. The product was tosylated without any further purification. The tosylated product showed two spots on t.l.c. (solvent C) R_F 0.73 (major), 0.70 (minor) and they were assigned as allo (135) and gluco (136) isomers respectively. The total radiochemical yield from (131) \rightarrow (135,136) was 94.4% (see appendix, page 138 for detailed calculation). Since the gluco derivative (136) is resistant⁴² to displacement reaction with charged nucleophile (X^-), the product was not purified any further and treated with tetrabutylammonium fluoride in acetonitrile. The fluoride (137) was isolated by p.l.c. (solvent F) as a mobile syrup and hydrolyzed to $^3H-(C_3)$ -3-fluoro-D-glucose (138) by Amberlite IR 120 (H^+). The overall radiochemical yield from (131) \rightarrow $^3H-(C_3)$ -3-deoxy-3-fluoro-D-glucose (138) was 57.1% (see appendix, page 138 for calculation).

CHAPTER III

EXPERIMENTAL

General

All melting points were determined on a Fischer-Johns apparatus and are uncorrected. All solvent removal, unless otherwise stated, was carried out under reduced pressure using a Buchi rotary evaporator, at a water bath temperature not exceeding 40°.

All reagent grade chemicals were used without further purification unless so specified. All pyridine used was anhydrous, and was dried by shaking with potassium hydroxide pellets. Methanol was dried when necessary by refluxing with magnesium and iodine followed by distillation. Dimethylformamide (DMF) and acetonitrile were dried by distillation over phosphorous pentoxide.

All micro-analyses were determined by A.B. Gygli Micro-analysis Laboratories Limited, Toronto, Ontario. The specific rotation values quoted were measured with a manual polarimeter (Rudolph and Sons Inc., N.J., U.S.A.) using the sodium emission D-line ($\lambda=589\text{ m}\mu$) and 2 dm tube.

Spectroscopy

The infra red (i.r.) spectra were recorded over ranges of 4000-650 cm^{-1} using a Beckman IR-12 spectrophotometer. Intensities were coded as follows: w=weak (100-75% transmission), m = medium (74-40% transmission) and s = strong

(39-0% transmission). Nuclear magnetic resonance spectra were recorded on a JEOLCO-C-60HL n.m.r. spectrometer.

The chemical shifts are expressed in parts per million (δ) downfield from the internal standard, tetramethylsilane (T.M.S.). The splitting pattern of each resonance is reported using the following code system: s = singlet; d = doublet, t = triplet, m = multiplet, cm = complex multiplet, and b = broad. ^{19}F chemical shifts were measured relative to trifluoroacetic acid (T.F.A) as a external standard using a field sweep with range of 90 ppm. Electron impact (high resolution) and field desorption mass spectra were recorded on a varian MAT CH5-DF spectrometer.

Chromatography.

Thin layer chromatography (t.l.c.) was carried out on 20x20 cm plastic plates, coated with a 0.2 mm layer of silica gel (Brinkmann). After application of the materials, using glass capillaries, the plates were developed in the solvent specified, and the carbohydrate compounds were detected by spraying with a 50% solution of concentrated sulphuric acid in ethanol, followed by heating at 110° for 2-5 min.

Preparative thin layer chromatography (p.l.c.) was performed using 40x20 cm and 20x20 cm glass plates, coated with a 1.2 mm layer of silica gel PF 254, which were dried overnight and activated by heating at 110° for 1 hour.

A 25% solution of a mixture under treatment, in a suitable non-polar solvent, was applied repeatedly as a continuous thin band along one 40 cm edge of the chromatoplate. Developed chromatoplates were air dried and visualized by exposure to U.V. radiation (254) and bands corresponding to the desired component were marked and removed from the absorbent by elution and filtration using suitable solvent, followed by removal of the solvent under reduced pressure.

The following solvent systems were used in t.l.c. and p.l.c. techniques.

- (A) Ethyl Acetate
- (B) Ethyl Acetate : Ethyl alcohol / 95 : 5
- (C) Ethyl Acetate : Ethyl alcohol / 4 : 1
- (D) Ethyl Acetate : Petroleum Ether (30-60) / 3 : 1
- (E) Ethyl Acetate : Petroleum Ether (30-60) / 1 : 1
- (F) Ethyl Acetate : Petroleum Ether (30-60) / 1 : 3
- (G) Ether : Petroleum Ether (30-60) / 6 : 4
- (H) Ether : Petroleum Ether (30-60) / 7 : 8
- (I) Benzene : Methyl alcohol / 95 : 5
- (J) Petroleum Ether : Methyl alcohol / 95 : 5
- (K) Ethyl Acetate : Acetic Acid : Water / 3 : 3 : 1
- (L) Ethyl Acetate : Methyl Alcohol / 10 : 1
- (M) Ethyl Alcohol : Water : Ammonium hydroxide / 80 : 16 : 4
- (N) Isopropyl Alcohol : Ethyl Acetate : Water : Acetic Acid / 83 : 11 : 5 : 1

Descending paper chromatography was carried out on Whatmann No. 1 paper. Microcrystalline cellulose t.l.c. was used occasionally as a rapid and convenient substitute for paper chromatography using the same solvent system.

The following solvent systems were used in paper chromatography and microcrystalline cellulose t.l.c.

- (O) n-Butanol : Water : Acetic Acid / 2 : 1 : 1
- (P) n-Butanol : Benzene : Formic acid : Water /
100 : 19 : 10 : 25
- (Q) n-Butanol : Ethyl alcohol : Water / 10 : 3 : 6
- (R) Ethyl Acetate : Acetic Acid : Water / 3 : 1 : 3
- (S) n-Butanol : Ethyl alcohol : Water / 4 : 1 : 5

Sugar epoxides¹²¹ were detected by spraying the developing chromatograms with a solution of methyl red (0.01 g) and sodium iodide (5.0 g) in n-butanol (100 ml) and heating at 110° for 2-5 min. Carbohydrate derivatives containing vicinal diol groups were detected by spraying the chromatograms with a 1% solution of sodium metaperiodate in acetone, followed by benzidine spray¹²². Reducing sugars were detected using aniline hydrogen phthalate spray¹²³. Lactones and acids were detected by hydroxylamine-ferric chloride¹²⁴ spray.

Column-chromatographic separations are described where they occur in the experimental section.

Radioactive Measurements

Radioactivities of tritium samples were measured on a liquid scintillation counter (Nuclear Chicago-Unilux II) using Bray's solution (New England Nuclear) as a cocktail mixture. Free sugars were dissolved in water. Sugar derivatives were dissolved in toluene before adding cocktail mixture.

Fischer-Kiliani reaction of 3-deoxy-3-fluoro-D-arabinose⁶³

To a frozen solution sodium cyanide (329 mg) in water (34 ml) in a glass stoppered tube, was added a solution of 3-deoxy-3-fluoro-D-arabinose⁶³ (106) (1 g) in 0.2 molar sodium bicarbonate (34 ml). The mixture was thawed and stored at room temperature for three days and reaction was monitored by t.l.c. (solvent A). The mixture was heated on steam bath for 3-4 hours (until the evolution of ammonia ceased). The mixture was concentrated under reduced pressure to a suitable volume (10 ml) and passed through a column containing 60 ml of Amberlite IR 120 (H^+) cation exchange resin. The effluent was concentrated under reduced pressure to a convenient volume, and a solution of barium hydroxide (saturated) was added in a quantity sufficient to produce a permanent pink colour with phenolphthalein. The solution was then acidified by means of a stream of carbon dioxide gas and the suspension was filtered. The filtrate was concentrated under reduced pressure to a thick

syrup which was dissolved in the minimum amount of 50% aqueous methanol and allowed to stand for a week at 4°. The mother liquor was removed from the crystals by means of pipette and the crystals were washed, in place, with ice-cold, 25% aqueous methanol.

The crystals were dissolved in minimum amount of water and passed through a column containing 40 ml of Amberlite IR-120 (H^+). The effluent was freeze-dried and then dried in vacuo over phosphorous pentoxide at 2 mm of Hg. Several attempts were made to crystallize the syrup without any success. The sample showed a single spot on paper chromatography (R_F 0.4, solvent S; R_F 0.5 solvent O) with extensive trailing and gave a positive test for lactones, carboxylic acid (ammonium hydroxide-ferric chloride spray), and was reducing to aniline-phthalate spray. i.r. (KBr) 3400 (s, OH), 2920 (m, C-H, alkane), 1745 (s, C=O) and 1630 (m, -C=C-) cm^{-1} .

The mother liquor was passed through Amberlite IR-120 (H^+) and effluent and washings were treated with decolorizing charcoal and evaporated to a thick syrup. It showed a single spot on paper chromatography (R_F 0.4, solvent S; R_F 0.5 solvent O) with extensive trailing and gave a positive test for presence of lactones and / or carboxylic acid (ammonium hydroxide-ferric chloride spray) and was reducing to aniline-phthalate spray. i.r. (KBr) 3400 (s, OH), 2920 (m, C-H, alkane), 1745 (s, C=O) and 1630 (m, -C=C-) cm^{-1} .

Sodium borohydride reduction of aqueous 50% methanol
insoluble fraction

To an ice-cold solution of sugar (275.0 mg) in water (10.0 ml) was added an ice-cold solution of sodium borohydride (60 mg) in water (5 ml). Temperature of the reaction mixture was maintained at 0-5⁰ and at pH 3-4 by addition of acetic acid externally. Total addition took one hour. After one and a half hours, the excess of sodium borohydride was destroyed by addition of acetic acid and solution was passed through Amberlite IR-120 (H⁺) (25 ml). The effluent and washings were collected and concentrated to a thick syrup which was dissolved in methanol and evaporated at reduced pressure. This process was repeated twice more to remove all boric acid as methyl borate. A trace amount of acetic acid was removed as an azeotropic mixture with toluene. The resulting syrup on t.l.c. (solvent L) was found to contain two major components, R_F 0.2 and R_F 0.45; one of which was found to be starting material (R_F 0.2) by comparative t.l.c. and i.r. spectrum. The product (R_F 0.45) was isolated by p.l.c. as a non-reducing syrup which crystallised on standing (25 mg).
i.r. (KBr) 3400 (s, OH), 1620 (m, -C=C-) cm.

Sowden-Fischer reaction of 3-deoxy-3-fluoro-D-arabinose.
4-deoxy-4-fluoro-1-deoxy-nitro-hexitol (110)

A suspension of 3-deoxy-3-fluoro-D-arabinose (2 g) in anhydrous methyl alcohol (4 ml) and nitromethane (7.2 ml)

was shaken with solution of sodium methoxide (0.42 g of sodium in 14 ml of anhydrous methyl alcohol). The mixture became noticeably cooler, the sugar dissolved rapidly and amorphous sodium salts began to precipitate. After shaking for 24 hours, the creamy yellow product was collected by filtration and washed with cold anhydrous methanol, ether (15 ml) and petroleum ether (b.p. 30-60, 15 ml) and dried over phosphorous pentoxide for one hour (yield 3.1 g). The sodium salt of the nitro alcohol was dissolved in an ice-cold water (25 ml) and immediately passed through a column containing 50 ml of Amberlite IR-120 (H^+). Concentration of the pale yellow effluent at reduced pressure yielded a viscous syrup (2.65 g). Fractional crystallization from ethyl alcohol gave the following fractions: Fraction I, 710 mg, m.p. 116° ; Fraction II, 335 mg, m.p. 116° . Both fractions were found to be chromatographically identical (t.l.c., R_F 0.55, Solvent B; paper chromatography R_F 0.41, Solvent P).

i.r. (KBr) 3300, (3520 (s, OH), 1540 (s, $-NO_2$) and 1060 (s, C-F) cm^{-1} . Anal. calc. for $C_6H_{12}O_6NF$: C, 33.9; H, 5.65; N, 6.52; F, 9.2;

found: C, 33.8; H, 5.6; N, 6.57; F, 8.9.

Hydrolysis of 4-deoxy-4-fluoro-1-deoxy-1-nitro-hexitol

(a) By Amberlite IR-120 (H^+)

4-deoxy-4-fluoro-1-deoxy-1-nitro-hexitol (185 mg) was dissolved in 2N sodium hydroxide (0.5 ml) and solution was kept standing for 10 minutes and Amberlite IR-120 (H^+) (10 ml) was added. The mixture was stirred for 15 minutes

at room temperature and filtered. The filtrate was shaken with Amberlite 45 (OH^-) to neutral pH for 10 minutes and filtered. The filtrate was concentrated at reduced pressure to a thick syrup which was crystallised by standing overnight (yield 95 mg, m.p. 105^0). The resulting crystalline material was found to be a mixture of 3-deoxy-3-fluoro-D-arabinose (major) and starting material (comparative i.r. and t.l.c.).

b) By sulphuric acid

An ice-cold solution of 4-deoxy-4-fluoro-1-deoxy-1-nitro-hexitol (600 mg) in 2N sodium hydroxide (0.8 ml) was added dropwise to a stirred solution of sulphuric acid (0.9 ml) in water (1.1 ml). The temperature of the reaction mixture was maintained at $10-15^0$. After 15 minutes the solution was passed through a column of Amberlite IR-120 (H^+) (20 ml) and then neutralized with barium carbonate to pH 7 and filtered. The filtrate was concentrated to a thick syrup at reduced pressure and absolute alcohol (40 ml) was added and the solution was filtered. The filtrate was evaporated to a thick syrup which was tentatively identified (i.r., comparative cellulose t.l.c.) as a mixture of 3-deoxy-3-fluoro-D-arabinose and 4-deoxy-4-fluoro-D-mannose (see t.l.c. plate 1 and 2, page 151).

Attempts to selectively benzylate methyl α -D-galactopyranoside¹⁰⁸ (113).

To a solution of methyl α -D-galactopyranoside¹⁰⁸ (1 g) in anhydrous dimethylformamide (10 ml) was added silver oxide (6 g) and benzyl bromide (6 ml). The reaction mixture

was stirred for three days at room temperature and worked up in the usual manner. The resulting syrup on t.l.c. (solvent J) showed the presence of at least three components (R_F 0.5, 0.33 and 0.12). Components with R_F 0.5 and 0.33 were found to be non-sugar impurities and they were separated by p.l.c. However, components with R_F 0.12, when applied on t.l.c. (solvent E) showed mixture of more than 7 components. The same results were obtained when benzylation was done at 4° by following the above mentioned procedure.

Mesylation of methyl tri-O-benzyl- α -D-galactopyranoside

The above crude product (R_F 0.12, solvent J) (1 g) was dissolved in anhydrous pyridine (10 ml) and mesyl chloride (0.31 g) was added. The reaction was protected from moisture and stirred for 24 hours. Then it was poured into ice-cold water (100 ml) and the product was extracted with chloroform (2x50 ml). The chloroform layer was washed with water, 1N hydrochloric acid, saturated sodium bicarbonate and finally with water, and after drying ($MgSO_4$), was evaporated to a thick syrup which showed on t.l.c. the presence of at least three components (R_F 0.15, 0.2 and 0.30; solvent G). The components were separated by p.l.c. (solvent G) and showed similar i.r. (absence of OH group) and n.m.r. spectrum. Anal. calc. for $C_{29}H_{34}O_8S$: C, 64.2 H, 6.3; S, 5.9
found: C, 64.8; H, 6.4; S, 4.9.

Methyl α -D-galactopyranoside¹⁰⁸ (113)

Reagent grade, anhydrous D-galactose (200 g) was boiled vigorously under reflux with a solution of hydrogen chloride in anhydrous methanol (2% w/v, 1600 ml) until it dissolved after which the solution was refluxed for 7 hours. The cool solution was shaken with lead carbonate (220 g) for three hours (to pH 7). Lead salts were removed with the aid of Kieselguhr and washed with methanol (100 ml) and the total filtrate was evaporated to an amber syrup. The warm syrup was mixed with water (60 ml) and rapidly deposited coarse crystals of methyl α -D-galactopyranoside monohydrate. Crystallization of the title compound was complete after 24 hours at room temperature. The syrupy mother liquor was removed from the crystals by suction and resulting solid was washed by carefully stirring it under gentle suction, with a small portion of 80% ethanol (100 ml, chilled to 5°), followed by absolute ethanol (2x32 ml). The air dried solid (99 g, 46%) was used for the next reaction without any further purification. m.p. 90-98° (α)_D²³ + 176° (c 2.0, H₂O). (Lit.¹⁰⁸ m.p. 99-113°, (α)_D²³ + 178°).

Methyl 2,3,6-tri-O-benzoyl- α -D-galactopyranoside¹⁰⁵ (114)

A magnetically stirred solution of methyl α -D-galactopyranoside monohydrate (14.84 g) in anhydrous pyridine was

(450 ml) cooled by means of a solid carbon dioxide-acetone bath at -40° . Benzoyl chloride (33.9 ml, 4.2 molar ratio) was added dropwise (30-60 min) with exclusion of moisture. The bath temperature was kept between -30 to -40° for three hours and then 4° for 20 hours. The reaction mixture was stirred for two more days at room temperature. Pyridine was removed at reduced pressure below 40° and the residue was dissolved in chloroform (300 ml). The chloroform solution was washed successively with 2N hydrochloric acid (until free from pyridine), a saturated solution of sodium bicarbonate (200 ml) and finally with water (200 ml), and was dried (MgSO_4). Removal of solvent gave a syrupy mixture which crystallized on standing overnight at room temperature. Recrystallization from 90% aqueous ethanol (60 ml) gave the title compound (23.2 g, 65%) as a colourless solid m.p. $137-138^{\circ}$, $(\alpha)_D^{23} + 122^{\circ}$ (c 1.5, chloroform). (Lit¹⁰⁵ m.p. $139-140^{\circ}$, $(\alpha) + 123^{\circ}$).

Methyl 2,3,6-tri-O-benzoyl-4-O-methylsulphonyl - α -D-galactopyranoside¹⁰⁷ (115)

To a solution of methyl 2,3,6-tri-O-benzoyl- α -D-galactopyranoside (30 g) in dry pyridine (120 ml) was added methylsulphonyl chloride (15 ml). The reaction was stirred at room temperature for 24 hours while protected from moisture. Then the reaction mixture was poured into water (200

ml) and extracted with chloroform (350 ml). The chloroform layer was washed with 2N hydrochloric acid (3x300 ml) followed by saturated sodium bicarbonate (300 ml), and finally with water (300 ml) then dried (MgSO_4) and evaporated to dryness in vacuo to give a thick syrup which crystallised on standing overnight. Recrystallisation from absolute alcohol gave the desired product (29 g, 82%). m.p. 142° (Lit¹⁰⁷ m.p. $141-142^\circ$). i.r. (CHCl_3) 1785 (s, -C=O), 1355 , 1275 (s, C-O-C), 1180 (m, -SO_3) cm^{-1} . n.m.r. data $8.2-7.1$ (m, 15H , $\text{-C}_6\text{H}_5$), 6.5 (m, 4H , C_1H , C_2H , C_3H , C_4H), $4.75-4.25$ (m, 3H , C_5H , C_6H_2), 3.45 (s, 3H , -OCH_3), 3.15 (s, 3H , $\text{-SO}_3\text{CH}_3$).

Methyl 4-O-methylsulphonyl- α -D-galactopyranoside¹⁰⁷ (116)

A suspension of methyl 2,3,6-tri-O-benzoyl-4-O-methylsulphonyl- α -D-galactopyranoside (46 g) in anhydrous methanol (300 ml) was cooled to 4° and a solution of sodium methoxide (1.5 g of sodium in 25 ml of anhydrous methanol) was added. The reaction was kept stirring for 24 hours at 4° , then neutralized with Amberlite IR-120 (H^+) (60 ml). The filtrate was evaporated to dryness in vacuo. The residue was partitioned between 150 ml each of chloroform and water. The water layer was washed with 50 ml of chloroform and treated with decolourizing charcoal, and evaporated in vacuo to give a solid residue. Recrystallization from absolute alcohol

(80 ml) gave 11 g of the product; m.p. 162-163⁰. The ethanol filtrate was cooled to -40⁰ and then brought to room temperature to give second crop of the product (5 g). m.p. 162-163⁰; admix m.p. 162-163⁰ (Lit.¹⁰⁷ m.p. 159-161⁰) (total yield 16 g; 75%).

i.r. (KBr) 3460, 3430, 3370, (s, -OH), 1185 (s, -SO₃) cm⁻¹.
n.m.r. data 5.1 (d, J = 3, 1H, C₁H), 4.8 (s, 3H, C₂OH, C₃OH, C₆OH), 4.15 (t, J = 3, 1H, C₄H), 4.1-3.65 (m, 5H, C₂H, C₃H, C₅H, C₆H₂), 3.5 (s, 3H, -OCH₃), 3.33 (s, 3H, -SO₃CH₃).

Methyl 2,3,6-tri-O-benzyl-4-O-methylsulphonyl- α -D-galactopyranoside (117)

To a solution of methyl-4-O-methylsulphonyl- α -D-galactopyranoside (15 g) in anhydrous dimethyl formamide (130 ml) was added silver oxide (39 g) and benzyl bromide (36 ml). The reaction mixture was protected from moisture and stirred magnetically for three days at room temperature. Then it was filtered and washed with chloroform (3x25 ml). The filtrate was diluted with chloroform (250 ml) and filtered. The filtrate was washed with water (3x200 ml). Pyridine (30 ml) was added to the chloroform layer and the solution was washed successively with water (2x250 ml), 2N hydrochloric acid (3x100 ml), saturated sodium bicarbonate (100 ml) and finally with water (2x200 ml). The chloroform extract was dried (MgSO₄) and evaporated to dryness in vacuo. The resulting residue (32 g) was divided into two parts and column-

chromatographed over 450 g of aluminium oxide (Brinkmann, activity 11). Elution with petroleum ether (b.p. 30-60): ether (100:20 v/v) (600 ml) removed dibenzyl ether (6 g). Elution with ether (500 ml) yielded the title compound (21.5 g, 72%) as a colourless syrup. R_F 0.47 (solvent E); $(\alpha)_D^{23} + 43^0$ (c 2.03, chloroform). i.r. (CHCl_3) 3040, 3070, 3090 (m, $-\text{C}_6\text{H}_5$), 1600-2000 (w, $-\text{C}_6\text{H}_5$), 1500, 1450 (m, $-\text{C}_6\text{H}_5$), 1175 (s, $-\text{SO}_3$), 710-790 (m, C_6H_5) cm^{-1} . n.m.r. data 7.25 (s, 15H, C_6H_5), 5.25 (d, $J = 2.5$ Hz, C_1H), 4.75-4.45 (m, 6H, $\text{CH}_2-\text{C}_6\text{H}_5$), 4.1-3.5 (m, 6H, C_2H , C_3H , C_4H , C_5H , C_6H_2), 3.35 (s, 3H, $-\text{OCH}_3$), 2.9 (s, 3H, $-\text{SO}_3-\text{CH}_3$). Anal. calc. for $\text{C}_{29}\text{H}_{34}\text{O}_8\text{S}$: C, 64.2; H, 6.3; S, 5.9; found: C, 64.1; H, 6.4; S, 5.8.

Methyl 2,3,6-tri-O-benzyl-4-deoxy-4-fluoro- α -D-glucopyranoside (118)

Tetrabutylammonium fluoride⁴³ was prepared by titration of 40% aqueous tetrabutylammonium hydroxide (60 ml) with 50% aqueous hydrofluoric acid to pH 7.0. The solution was concentrated under reduced pressure, and the resulting syrup was dried and stored overnight over phosphorus pentoxide at 0.1 mm.

A mixture of methyl 2,3,6-tri-O-benzyl-4-O-methylsulphonyl- α -D-galactopyranoside (7.9 g), tetrabutylammonium fluoride (freshly prepared) and anhydrous acetonitrile (70 ml) was kept under gentle reflux at 70-80^b, and reaction was

monitored by t.l.c. (solvent E). After 3 days the reaction appeared to be complete, and the mixture was poured into water (50 ml) and extracted with ether (3x150 ml). The ethereal layer was dried (MgSO_4) and concentrated under diminished pressure. The syrupy residue was submitted to column chromatography on silica gel (grade H, mesh size 60-200, 240 g). Elution with ether:petroleum ether (b.p. 30-60) (70:30 v/v) yielded methyl 2,3,6-tri-O-benzyl-4-deoxy-4-fluoro- α -D-glucopyranoside (5g, 73%) as a colourless syrup. R_F 0.63 (solvent G), 0.68 (solvent A); $(\alpha)_D^{23} + 48.8^\circ$ (c 1.23, chloroform). i.r. (CHCl_3) 3020, 3070, 3090 (m, $-\text{C}_6\text{H}_5$), 1600-2000 (w, $-\text{C}_6\text{H}_5$), 1500, 1450 (m, $-\text{C}_6\text{H}_5$), 1050 (s, C-F), 710-790 (m, $-\text{C}_6\text{H}_5$) cm^{-1} . n.m.r. data 7.3 (s, 15H, $-\text{C}_6\text{H}_5$), 5.05-3.5 (m, 13H, C_1H , C_2H , C_3H , C_4H , C_5H , C_6H_2 , $\text{CH}_2-\text{C}_6\text{H}_5$), 3.4 (s, $-\text{OCH}_3$). Anal. calc. for $\text{C}_{28}\text{H}_{31}\text{O}_5\text{F}$: C, 72.1; H, 6.65; F, 4.16; found: C, 72.00; H, 6.69; F, 4.16.

Methyl 4-deoxy-4-fluoro- α -D-glucopyranoside (119)

A solution of methyl 2,3,6-tri-O-benzyl-4-deoxy-4-fluoro- α -D-glucopyranoside (4.9 g) in ethanol (85 ml) was shaken in the presence of hydrogen and palladised charoal (5g, 5%) at room temperature until hydrogen uptake ceased (24 hours). The reaction mixture was filtered and concentrated under reduced pressure. The residue was dissolved in the minimum amount of methyl alcohol and placed on a column of silica gel (grade H, mesh size 60-200, 70 g) and eluted with ethyl acetate (600 ml). After removal of the solvent under reduced pressure it crystal-

lized (1.1 g, 53%). Recrystallization from ethyl acetate-acetone (1:1) gave the title compound as a colourless compound.

m.p. 129-130°, R_F 0.3 (solvent A), 0.44 (solvent C); $(\alpha)_D^{23} + 1310$ (c 0.92, water).

i.r. (KBr) 3380, 3460 (s, -OH), 1035 (s, C-F) cm^{-1} .

n.m.r. data 4.8-4.55 (m, 3H, $\text{C}_2\text{-OH}$, $\text{C}_3\text{-OH}$, $\text{C}_6\text{-OH}$), 4.2-3.5 (m, 5H, C_1H , C_2H , C_3H , C_4H , C_5H), 3.4 (s, 3H, $-\text{OCH}_3$) and 3.15 (s, 2H, C_6H_2).

Anal. calc. for $\text{C}_7\text{H}_{13}\text{O}_5\text{F}$: C, 42.85; H, 6.63; F, 9.69;

found: C, 42.68; H, 6.71; F, 9.43.

4-deoxy-4-fluoro-D-glucose (120).

A solution of methyl 4-deoxy-4-fluoro- α -D-glucopyranoside (500 mg) in 2M sulphuric acid (50 ml) was gently refluxed for 3 hours and the reaction was monitored by t.l.c. (solvent C). After neutralisation with solid barium carbonate, the reaction mixture was filtered and concentrated to dryness under reduced pressure. The residue was taken up in absolute alcohol and the solution was filtered through a bed of Kieselguhr and decolorizing charcoal and evaporated to dryness. The syrupy residue crystallized on standing at room temperature. Recrystallization from ethanol-ethyl acetate gave the title compound (330 mg, 71%) as a colourless solid. m.p. 189-190°.

$(\alpha)_D^{23} + 26.5$ 10 min $+ 50^\circ$ (c 1.0, 24 hours, equil., water).

(Lit⁶⁷ m.p. 187-189°, $(\alpha)_D^{23} + 26$ 9 min 49° (76 hours equil., water), and mixed m.p. with authentic⁶⁷ sample* was 188-189° R_F 0.17

*kindly supplied by Prof. N.F. Taylor

(solvent C) and gave positive reaction to aniline phthalate spray.

i.r. of the sample was identical to the authentic sample⁶⁷.

Anal. calc. for $C_6H_{11}O_5F$: C, 39.5; H, 6.0; F, 10.4;

found: C, 39.34; H, 6.12; F, 10.32.

Methyl 2,3,6-tri-O-acetyl-4-deoxy-4-fluoro- α -D-glucopyranoside
(121).

To methyl 4-deoxy-4-fluoro- α -D-glucopyranoside (200 mg) in ice-cold pyridine (5 ml), acetic acid (0.5 ml) was added. The solution was kept 24 hours at room temperature, poured into ice-water (15 ml), and product extracted with chloroform (3X10 ml). The chloroform extract was washed successively with 2N hydrochloric acid (3X10 ml), saturated sodium bicarbonate (10 ml), and finally with water (10 ml). Then the chloroform extract was dried ($MgSO_4$), filtered, and evaporated to dryness, leaving methyl 2,3,6-tri-O-acetyl-4-deoxy-4-fluoro- α -D-glucopyranoside as a colourless viscous syrup. R_F 0.2

(solvent A) $(\alpha)_D^{23} + 41.0^0$ (c 0.55, chloroform)

i.r. ($CHCl_3$) 1730 (s, C=O), 1275-1320 (s, C-O-C) cm^{-1}

n.m.r. (C_6D_6) data three pairs of doublet at 5.4-6.0 (1H, $J_{4,5} = 9.5$ Hz, $J_{4,3} = 9.5$ Hz, $J_{4,F} = 3.75$, C_4H), 4.65 (t, 1H, C_1H), 4.6-4.1 (cm, 2H, C_6H_2), 4.1-3.9 (cm, 1H, C_5H), 3.7 (t, $J = 9.0$, C_3H), 3.55-3.3 (cm, 1H, C_2H) 2.65 (s, 3H, -OCH₃), 1.45 (d, $J = 4.5$, 9H, -C(=O)-CH₃)

Anal. calc. for $C_{13}H_{19}O_8F$: C, 48.5; H, 5.9; F, 5.9

Found: C, 4.84 ; H, 5.86; F, 5.69

Electron impact mass spectrum of the title compound contains the following peaks expressed as m/e (% abundance, using m/e 160 as the base peak).

28 (>100), 29 (19), 31 (5), 32 (40), 39 (8), 40 (5), 41 (12), 42 (19), 43 (>100), 44 (42), 45 (11), 51 (7), 53 (5), 55 (12), 57 (19), 59 (14), 60 (10), 61 (11), 69 (13), 70 (25), 71 (5), 72 (23), 79 (6), 81 (10), 82 (5), 85 (6), 87 (11), 94 (7), 97 (13), 98 (15), 99 (32), 100 (73), 101 (41), 102 (73), 103 (53), 105 (54), 107 (23), 108 (9), 112 (13), 117 (11), 118 (8), 119 (13), 121 (51), 122 (5), 127 (5), 129 (15), 131 (18), 139 (22), 140 (10), 141 (13), 142 (17), 143 (6), 144 (33), 145 (19), 149 (26), 159 (5), 160 (100), 161 (21), 169 (30), 182 (8), 189 (11), 194 (8), 202 (23), 203 (64), 204 (6), 231 (20), 242 (18), 249 (7), 291 (5).

Methyl 2,3,6-tri-O-benzoyl-4-deoxy-4-iodo- α -D-gluco- and -galactopyranoside (122,123).

A mixture of methyl 2,3,6-tri-O-benzoyl-4-O-methyl-sulphonyl- α -D-galactopyranoside (10 g) and sodium iodide (12.8 g) were refluxed for one hour in anhydrous dimethyl formamide (140 ml). The reaction mixture was cooled and diluted with ether (250 ml). The ethereal solution was washed successively with water (4x200 ml). The ether layer was dried ($MgSO_4$) and evaporated to a thick syrup, which was shown by t.l.c. (solvent H) to contain at least two products and some unreacted

starting material. The syrup was divided into two parts and column chromatographed over 190 g of silica gel. Elution with ether-petroleum ether (b.p. 30-60) (2:5, v/v) yielded 500 mg of the colourless thick syrup which was identified by n.m.r. as methyl 2,3,6-tri-O-benzoyl-4-deoxy-4-iodo- α -D-galactopyranoside (123). R_F 0.51 (solvent H).

i.r. (CHCl_3) 3050 (w, $-\text{C}_6\text{H}_5$), 1730 (s, $\text{C}=\text{O}$), 1280 (s, $\text{C}-\text{O}-\text{C}$) cm^{-1} .

n.m.r. data 8.3-7.2 (m, 15H, C_6H_5), 6.4-4.9 (cm, 7H, C_1H , C_2H , C_3H , C_4H , C_5H , C_6H_2), 3.5 (s, 3H, $-\text{OCH}_3$)

Elution with ether-petroleum ether (b.p. 30-60) (3:5 v/v) gave a colourless light solid which was redissolved in benzene and evaporated to dryness. Recrystallization from absolute alcohol gave 5.5 g of the gluco-isomer (122).

m.p. 159° , $(\alpha)_D^{23} +110^\circ$ (c 1.55, CHCl_3), (Lit¹¹² m.p. $158-160^\circ$ $(\alpha)+112^\circ$) R_F 0.46 (solvent H)

i.r. 3050 (w, $-\text{C}_6\text{H}_5$), 1730 (s, $\text{C}=\text{O}$), 1280 (s, $\text{C}-\text{O}-\text{C}$) cm^{-1}

n.m.r. data 8.3-7.25 (m, 15H, $-\text{C}_6\text{H}_5$), 6.2 (t, $J = 9.5$, C_4H),

5.3 (s, 1H, C_1H), 5.15 (t, 1H, $J_{2,1} = 4$ $J_{2,3} = 5$, C_2H),

5-4.1 (cm, 4H, C_3H , C_5H , C_6H_2), 3.5 (s, 3H, $-\text{OCH}_3$).

Anal. calc. for $\text{C}_{28}\text{H}_{25}\text{O}_8\text{I}$: C, 54.56; H, 4.09; I, 20.59;

found: C, 54.81; H, 4.35; I, 20.39.

Elution with ether gave 600 mg of the starting material (comparative t.l.c., i.r., n.m.r. and m.p.)

Methyl 2,3,6-tri-O-benzoyl-4-deoxy- α -D-glucopyranoside¹¹² (124)

A solution of sodium acetate (3 g) in alcohol (3.1 ml of water and 31 ml of absolute alcohol) was added to a mixture of methyl 2,3,6-tri-O-benzoyl-4-deoxy-4-iodo- α -D-glucopyranoside (4.5 g) in ethyl acetate (62 ml). The mixture was shaken in the presence of hydrogen and palladised charcoal (2 g, 5%) at room temperature until hydrogen uptake ceased (13 hours). The reaction mixture was filtered and evaporated to dryness. The resulting residue was taken up in 125 ml of benzene and washed successively with water (100 ml), a saturated solution of sodium bicarbonate (50 ml), 20% sodium thiosulphate (50 ml) and finally with water (100 ml). The benzene layer was dried and evaporated to a thick syrup which crystallized on standing. Recrystallization from absolute alcohol gave colourless needle shaped crystals (5.2 g).

R_F 0.44 (solvent H), m.p. 116-117⁰, $(\alpha)^{23}_D +137$ (c. 1.1, CHCl₃).

(Lit¹¹² m.p. 116-117⁰, $(\alpha)^{23}_D +133$).

n.m.r. data 8.2-7.2 (m, 15H, C₆H₅), 5.8 (sextet, 1H, J = 4.5, C₁H), 5.4 (d, 1H, J = 3, C₂H), 5.2 (d, 1H, J = 3, C₃H), 4.6-4.4 (m, 3H, C₅H, C₆H₂), 3.5 (s, 3H, -OCH₃), 2.7-2.3 (m, 1H, C₄H_{eq}), 2.0 (t, 1H, J = 10.5, C₄H_{ax}).

Anal calc. for C₂₈H₂₆O₈: C, 68.56; H, 5.34

found: C, 68.38; H, 5.21.

Methyl 4-deoxy- α -D-glucopyranoside¹¹² (125)

A suspension of methyl 4-deoxy-2,3,6-tri-O-benzoyl- α -D-glucopyrano-

side (2.0 g) in anhydrous methanol (40 ml) was cooled to 4° and an ice-cold solution of sodium methoxide (75 mg of sodium in 1.5 ml of anhydrous methanol) was added. The reaction mixture was stirred for 20 hours at 4°; then neutralized with Amberlite IR-120 (H⁺) (20 ml) and filtered. The filtrate was evaporated in vacuo. The residue was dissolved in the minimum amount of methyl alcohol and column chromatographed over 40 g of silica gel. Elution with ethyl acetate-ethanol (90:10) gave a chromatographically pure compound.

Recrystallization from ethyl acetate gave 560 mg (76%) of the title compound. R_F 0.32 (solvent C), m.p. 98-91°, (α)_D²³ + 176° (c 1.2, methanol): (Lit¹¹² m.p. 90-91°, (α)_D²³ + 175°).

4-deoxy-D-glucopyranose^{112,113,114,115} (126)

A solution of 4-deoxy- α -D-methylglucopyranoside (500 mg) in water (30 ml) was gently refluxed with Amberlite IR-120 (H⁺) (20 ml) for 8 hours and the reaction was monitored by t.l.c. (solvent C). The reaction mixture was filtered and the filtrate was evaporated to a thick syrup. It was dissolved in 20 ml of absolute alcohol and filtered through a bed of decolourising charcoal and evaporated to dryness to yield a syrup which crystallized on standing (12 hours).

Recrystallization from ethyl acetate: methanol gave 280 mg (60%) of the title compound. R_F 0.24 (solvent C), m.p. 139-141°, (α)_D²³ + 38° equilibrium → +60° (c 1.2, H₂O), (Lit^{112,113,114}, (α) + 29° → +55.5°, +44° → +60°, +36° → +58°)

Methyl 2,3,6-tri-O-benzyl-4-deoxy-4-iodo- α -D-glucopyranoside and galactopyranoside (127, 128)

A mixture of methyl 2,3,6-tri-O-benzyl-4-O-methylsulphonyl- α -D-galactopyranoside (6 g) and sodium iodide (5 g) were refluxed for one hour in dimethyl formamide (60 ml). The mixture was taken up in CHCl_3 (150 ml) and washed successively with water, a saturated solution of sodium bicarbonate, water, 20% sodium thiosulphate and finally with water, the ether layer was dried (MgSO_4) and evaporated to a thick syrup. The resulting syrup on t.l.c. (solvent H) was found to contain three major products (R_F 0.85, 0.7, 0.6) and some unreacted starting material (R_F 0.45). When the mixture was submitted to p.l.c using solvent H, it was resolved into three bands; the middle band (R_F 0.7, 0.6) was found to contain desired product (2.8 g, 52.8%), $(\alpha)_D^{23} + 6.7^\circ$ (c 0.75, CHCl_3).

n.m.r. data 7.35 (s, 15H, $-\text{C}_6\text{H}_5$), 4.9 (s, 1H, C_1H), 4.8-4.5(m, 6H, $\text{CH}_2-\text{C}_6\text{H}_5$), 4.2-3.45 (cm, 6H, C_2H , C_3H , C_4H , C_5H , C_6H_2), 3.4 (s, 3H, $-\text{OCH}_3$).

Anal. calc, for $\text{C}_{28}\text{H}_{31}\text{O}_5\text{I}$: C 58.5; H, 5.4; I, 22.1

found: C, 59.31; H, 5.32; I, 21.53

1,2;5,6-di-O-isopropylidene- α -D-ribo-hexofuranos-3-ulose¹²⁰
(131)

To 1,2;5,6-di-O-isopropylidene- α -D-glucose¹¹⁹, (22 g) in anhydrous dimethylsulphoxide (100 ml), was added acetic

anhydride (33 ml) and the mixture was heated with stirring at 65-70° for 2.5 hours. The DMSO and acetic acid were removed at reduced pressure (bath temperature 50-60°) and water (2 ml) was added to the resulting syrup. On standing overnight at 4°, a crystalline product separated out. This was broken up with petroleum ether (b.p. 30-60), collected and washed thoroughly with petroleum ether (b.p. 30-60°). The product was chromatographically pure (solvent F) but had lower melting point than that quoted, (m.p. 90-93°, Lit¹¹⁹ m.p. 108-110°). i.r. (KBr) 3400 (s, -OH), 1780 (w, -C=O). Further purification by crystallization with acetone gave colourless needle shaped crystalline compound. m.p. 108-110°, i.r. (KBr) 3400 (s, -OH) and had no absorption at 1780 cm⁻¹.

³H-(C₃)-1,2;5,6-di-O-isopropylidene-α-D-allofuranose (133)

To an ice-cold solution (4°) of 1,2;5,6-di-O-isopropylidene-α-D-ribo-hexofuranos-3-ulose (2 g) in 80% ethanol (15 ml) was added ³H-sodium borohydride (13 mg, 100 mci). Within a few minutes the colour of the reaction mixture was changed to dark brown. The temperature of the reaction mixture was maintained at 4°. After 30 minutes excess of the sodiumborohydride (150 mg, cold) was added and the solution stirred for one more hour. The reaction mixture was poured into the water (30 ml) and extracted with ethyl acetate (8x20 ml). The com-

combined ethyl acetate extract was dried (MgSO_4) and evaporated under reduced pressure to a thick syrup which crystallised spontaneously. The product showed a single spot on t.l.c. with solvent F (R_F 0.17), but showed two spots (R_F 0.6, 0.46) with solvent C. It was used for the next reaction without any further purification.

$^3\text{H}-(\text{C}_3)-1,2;5,6\text{-di-O-isopropylidene-3-O-toluene-p-sulphonyl-}\alpha\text{-D-allofuranose (135)}$

To an ice-cold solution of $^3\text{H}-(\text{C}_3)-1,2;5,6\text{-di-O-isopropylidene-}\alpha\text{-D-allofuranose}$ (2 g) in anhydrous pyridine (25 ml) was added p-toluenesulphonyl chloride (2 g). The mixture was stirred at room temperature for 24 hours and then poured into ice-water (250 ml). The resultant precipitate was filtered and washed with several portions of an ice-cold water and dried in vacuo. Recrystallization from ethanol (95%) gave the title compound as a colourless solid (2.616g, 91.6% radiochemical yield from (131) to (135), (136); Sp. activity 14.5 mci/mmol). The carrier (250 mg) was dissolved in the mother liquor by heating over a steam bath to yield a second crop (271 mg) making the total radiochemical yield 94.4% from (131) to (135), (136). m.p. $120-121^\circ$; the product showed a single spot (R_F 0.42) on t.l.c. with solvent F, but showed two spots (R_F 0.73, major; 0.7, minor) with solvent C. The product was used for the next reaction without any further purification.

$^3\text{H}-(\text{C}_3)-1,2;5,6\text{-di-O-isopropylidene-3-deoxy-3-fluoro-}\alpha\text{-D-glucofuranose (137)}$

Tetrabutylammonium fluoride⁴³ was prepared by titration of 40% v/v aq. tetrabutylammonium hydroxide (27 ml) with 40% hydrofluoric acid to pH 7. The solution was concentrated under reduced pressure at room temperature, freeze dried at 0.3 mmHg for 12 hours and then stored over P_{205} overnight at 0.3 mmHg.

A mixture of $^3\text{H}-(\text{C}_3)-1,2;5,6\text{-di-O-isopropylidene-3-O-toluene-p-sulphonyl-}\alpha\text{-D-allo-}$ and glucofuranose (135, 136) (2.7 g, 90.14 mci), tetrabutylammonium fluoride (freshly prepared) and acetonitrile (25 ml) was refluxed gently for 3.5 days. The reaction mixture was poured into ether (40 ml) and washed with water (3x20 ml). The water extracts were re-extracted with ether (2x20 ml). The combined ether extract was dried (MgSO_4) and concentrated under reduced pressure. The resulting oily residue was dissolved in anhydrous ether (10 ml) and submitted to p.l.c. (solvent F, two 40x20 plates) to yield the chromatographically pure title compound (1.1 g) as a brown coloured oily syrup. R_F 0.88 (solvent F), 0.68 (solvent C).

$^3\text{H}-(\text{C}_3)\text{-3-deoxy-3-fluoro-}\alpha,\beta\text{-D-glucose (138)}$

$^3\text{H}-(\text{C}_3)-1,2;5,6\text{-di-O-isopropylidene-3-deoxy-3-fluoro-}\alpha\text{-D-glucose (1.1 g)}$ was dissolved in 95% ethyl alcohol (10 ml)

and water (50 ml) and was stirred at 60-70° with Amberlite IR-120 (H⁺) (20 ml) for 8 hours. Concentration of the filtered solution at reduced pressure gave the chromatographically pure title compound as a brown coloured syrup which crystallized by seeding, and was recrystallized from acetone. m.p. 105-106°, R_F 0.45 (solvent C). (total activity 54.6 mci, Sp. activity 14.7 mci/mmole; radiochemical yield 60.5%, from (135), (136) to (138))

PART 2

CHAPTER I

INTRODUCTION

The primary function of the human erythrocyte is to deliver oxygen to the tissues and transport carbon dioxide from the tissues to the lungs. The functions do not in themselves require the expenditure of metabolic energy. However to perform them efficiently, it is necessary for the red cell to carry a highly concentrated solution of haemoglobin while preserving the biconcave form of the cell. It must protect the membrane and haemoglobin from oxidative damage and prevent osmotic haemolysis. Preservation of the constituents of the red cell in an active form and the maintenance of ionic gradients across the cell membrane requires a source of metabolic energy¹²⁵.

The human erythrocyte appears to rely almost entirely upon hexoses, notably glucose, for the provision of the necessary energy. A specific uptake mechanism has developed to allow the rapid passage of monosaccharides, from the suspending medium to the cell interior. Since erythrocytes are easily obtained and prepared in a homogeneous suspension, the transport process has been extensively studied as a model system for application to other tissues.

An additional advantage of the red blood cell is its relative simplicity. It contains no nucleus, vacuoles, mitochondria, vesicles, granules, etc., normally found in other

cells. Kinetically this is important, since these cells form a two compartment system in which the membrane alone, (with no cellwall) separates the external and internal media. In addition, as the flux of glucose across the membrane, is up to 250 times faster than its metabolism, the passage of glucose is essentially independent of any other cellular function¹²⁷.

The only barrier to the flow of sugars into the erythrocyte is the membrane. The structure of this has been the subject of intensive study over many years¹²⁸. Despite this, it is obvious from reviews^{129, 130}, that there is conflict of opinion as to the detailed structure. There is however, general agreement on the essential features of the membrane. It is composed of a mixture of phospholipids and proteins, the latter existing as either α -helices or in the extended β -form¹³⁰. Recently, Singer and Nicolson¹³¹ have suggested a fluid mosaic model of membrane structure and proposed that it is applicable to most biological membranes. In their model, phospholipids are arranged as a discontinuous bilayer with ionic and polar heads in contact with water. The integral proteins are embedded and they span the entire membrane thickness with their ionic residues in contact with water. The peripheral proteins are partially embedded in, and partially protruding from, the membrane. The protruding parts have on their surfaces the ionic residues of

the proteins, while the non-polar residues are largely embedded. Partial purification¹³² of a membrane protein from human erythrocytes involved in D-glucose transport has been reported by Kahlenberg by using a variety of reagents capable of selectively extracting various membrane proteins. His results indicate that the D-glucose transport protein is an intrinsic component of the hydrophobic structure of the erythrocyte membrane and may be associated with the proteins of band 3 which are glycoproteins spanning the membrane bilayer.

Kozawa, in 1914, reported that isomeric sugars penetrated the red cell at different rates, and that methyl glucoside did not penetrate. This was the first evidence that simple diffusion did not explain the entrance of glucose¹³³. Massing, in the same year, reported the apparently contradictory evidence that, although glucose penetrated the cells freely, equilibrium was slow to be achieved¹³⁴. Further development of these observations was delayed for some years, until Orskov's group described an optical method for the study of these phenomena^{135,136}. As a result, a surge of interest took place in the post-war years.

LeFevre and his colleagues, argued that the uptake phenomenon was consistent with an active transport process^{137,138}. Later this hypothesis was abandoned when it was demonstrated

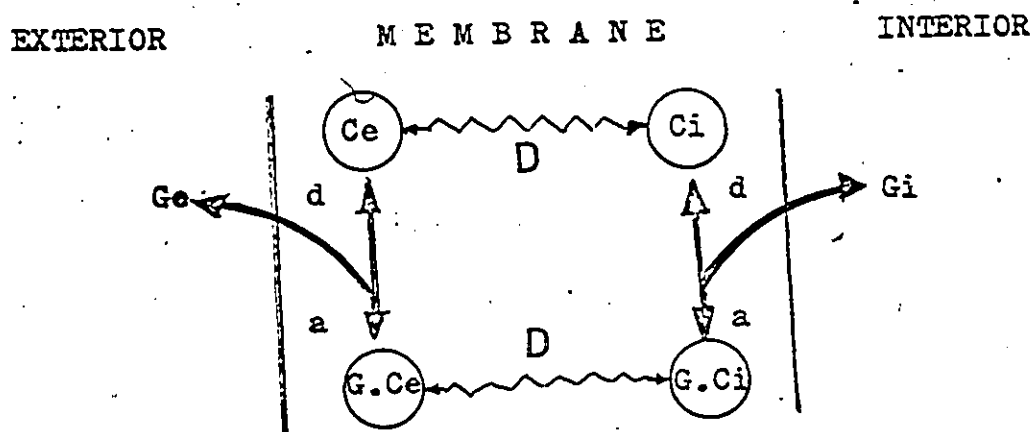
that glucose could flow outwards from the cells¹³⁹. At about the same time, Widdas had noticed a similar glucose transport process in sheep placenta and had proposed a simple carrier model¹⁴⁰. This he later applied to the erythrocyte and this model has remained, in one form or another, as a reasonably successful explanation of the observation¹²⁷.

The carrier-assisted passive transfer of material is referred to as facilitated diffusion and is independent of any energy supply. The proposed model^{127, 140, 141} described as facilitated diffusion or carrier mediated transport is shown schematically in figure 37. The formation of a carrier substrate complex promotes the high degree of specificity for particular sugars, and also accounts for the saturation kinetics which are observed. Since loaded carriers only move according to the concentration gradient, no metabolic energy is required and the reaction is fully reversible. This model has formed the basis of numerous investigations. The kinetic parameters of glucose transport have been determined by several methods, but different methods have often resulted in different absolute values of the parameters^{142, 143}. To account for this, Stein and his colleagues have proposed a modified carrier model which has a tetrameric subunit structure^{144, 145, 146, 147}. Hoare has developed an alternative argument which retains the principle of the monomeric carrier. His explanation suggested that the anomalous kinetic data can be explained by the rates of re-orientation of carriers which have just

Figure 37

THE CLASSICAL SYMMETRICAL MOBILE-CARRIER MODEL OF WIDAS¹⁶⁴
FOR FACILITATED DIFFUSION.

(Carrier C at either membrane interface reacts reversibly with substrate G in adjacent compartment to produce complex G.C; heavy arrows indicate relative rapidity of this association-dissociation (a,d) equilibrium. Both free carrier and complex diffuse (process D) within membrane between exterior and interior interface (denoted by subscript e,i). Relative slowness of transmembranal diffusion is indicated by resistance symbol in arrow denoting D process and by size of D symbol. Free mobility of carrier and complex across membrane is indicated by circular enclosures.)



released the substrate. The different methods used for determining the kinetic parameters could then give different values, if the re-orientation rates were altered by experimental conditions^{148, 149, 150}.

Inhibitors of glucose transport have been used in an attempts to identify functional groups on the carrier. Biphenyls, such as phloretin and stilbesterol, are good inhibitors^{151,152} as are the alkylating agents p-chloromercuribenzoate (PCMB) and 1-fluoro-2, 4-dinitrobenzene (FDNB)^{153,154,155}. Chlorpromazine at low concentration can stimulate glucose transfer¹⁵⁶. However no inhibition by metabolic inhibitor like fluoride has been observed. This has confirmed the independance of the carrier from a metabolic process¹⁵⁷.

The use of inhibitors has led to the detection of three different receptor groups ($-NH_2$, $-SH$, and imidazole) on the protein associated with glucose transport in the human erythrocyte¹⁵⁸. Also, there is evidence that phospholipids play an active part in the transport process^{159,160,161}. Phospholipase A2 treatment of ghost cells stimulated the uptake of glucose into stroma. This was interpreted as demonstrating that the carrier was protected by some part of the membrane¹⁶⁷. Certainly the carrier is deeply embedded in the membrane as is evidenced by the difficulty of its isolation¹⁶³ and the recent suggestion that it is a transmembrane protein¹³².

Undoubtedly the most active area of research, has been in the building of theoretical mathematical models and their testing by the determination of the kinetic parameters. Several such models have been proposed, with varying degrees of mathematical complexity¹⁶⁴. For the purposes of this work, the simplest and probably the best documented kinetic analysis has been described^{127, 165}.

The mechanism, upon which the kinetics are derived and shown schematically in Figure 38, may be described by following the movement of substrate X , from the bulk of the outer solution to the solution inside the cell, by the following steps:

Step 1. Diffusion of substrate X , from the bulk of the outer membrane surface, with a diffusion coefficient D_x .

Step 2. Combination of the substrate with carrier P , exposed at the outer surface, to form the complex PX , with a dissociation constant K_x .

Step 3. Movement of the complex, with a diffusion coefficient D_{px} , so that it becomes exposed to the inner membrane surface.

Step 4. Dissociation of the complex, with a dissociation constant K_x , releasing X at the inner surface.

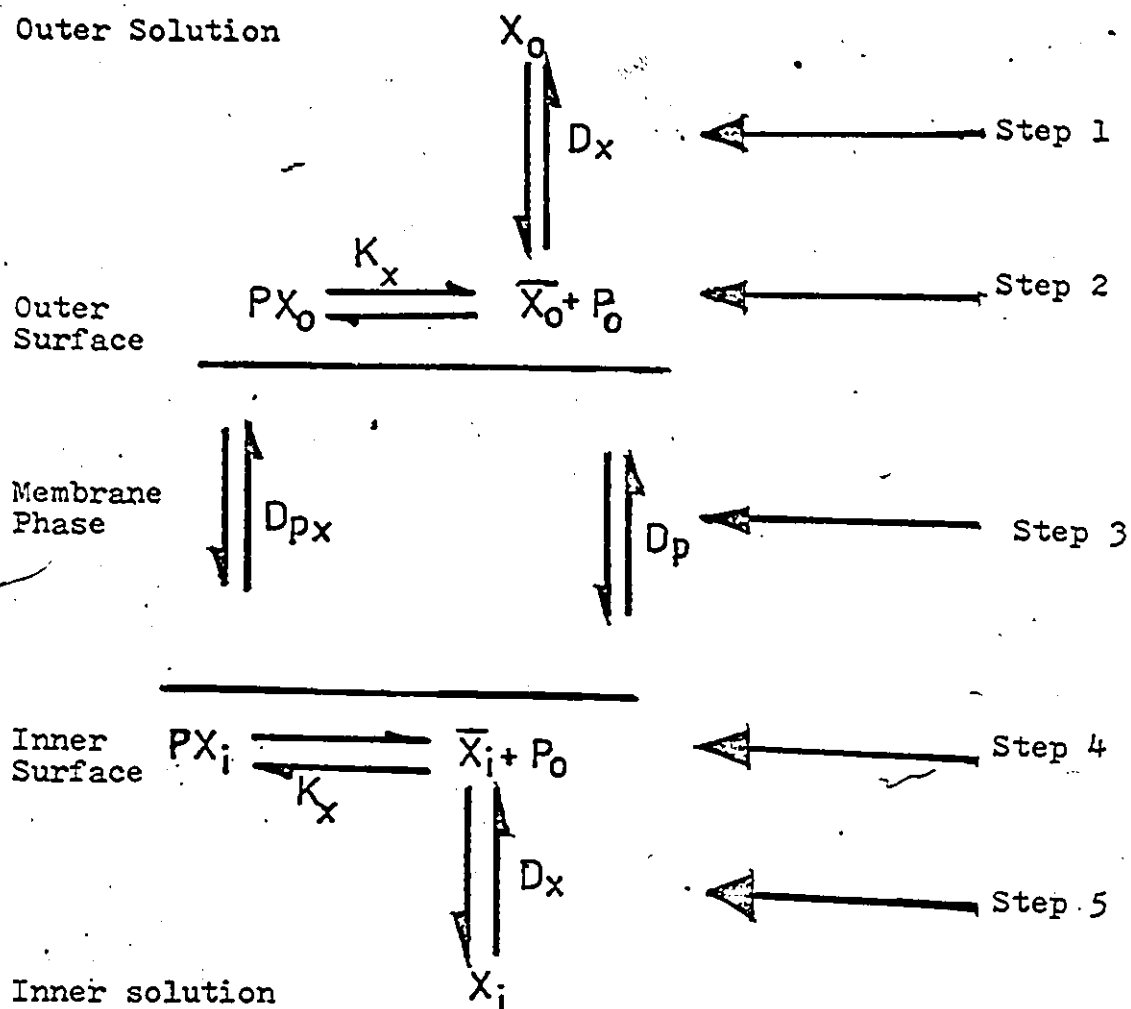
Step 5 Diffusion of X into the inner solution, with a diffusion coefficient D_x .

Each of these steps is reversible, so that outward movement of the sugar follows exactly the reverse pathway.

Figure 38

- A PROPOSED MATHEMATICAL CARRIER MODEL¹²⁶

(See text for explanation of the symbols)



The following assumptions are also made:

1. The free carrier P and the complex PX are confined to the membrane phase, and have the ability, in some unspecified way, to alternately expose themselves at one surface or the other. Free substrate is excluded from the membrane phase.
2. The rates of transfer of the complex and the free carrier, between the two membrane faces are equal, i.e. $D_{PX} = D_P$, and are the same whether transport is inward or outward.
3. The rates of diffusion of X in both solutions (steps 1 and 5), are very much greater than the overall transport rate, so that $(\bar{X}_0) = (X_0)$ and $(\bar{X}_1) = (X_1)$.
4. The rates of formation and dissociation of the complex (steps 2 and 4), are very much greater than the overall transport rate, so that the concentrations of P and PX, are at all times defined by the mass law equation:

$$K_x = \frac{(P) \cdot (X)}{(PX)}$$
5. The affinity of the substrate for the carrier is the same on both sides of the membrane (i.e. K_x has the same value at both the membrane surfaces).
6. The amount of carrier present in a given area of membrane remains constant.
7. The flux of complex or free carrier, in one direction across the membrane, is proportional to its concentration at the interface from which the flow originates. Thus inward flux

of complex equals $D_{px} \cdot (PX_o)$ and outward flux equals $D_{px} \cdot (PX_i)$ so that the net inward flux is $D_{px}((PX_o) - (PX_i))$ for the complex, and $D_p(P_o - P_i)$ for the carrier.

A kinetic equation may now be derived for the rate with which substrate X is transported into the cell.

From assumption 7, the basic rate equation is :

$$\frac{dx}{dt} = -D_{px} [(PX_o) - (PX_i)] \quad 1.$$

Since there is no way of directly determining either (PX_o) or (PX_i) , they must be expressed in terms of measureable quantities (X_o) and (X_i) .

The mass law equation is :

$$K_x = \frac{(X_i)(P_i)}{(PX_i)} = \frac{(X_o)(P_o)}{(PX_o)} \quad 2.$$

Therefore,

$$(P_i) = \frac{K_x(PX_i)}{(X_i)} \quad \text{and} \quad (P_o) = \frac{K_x(PX_o)}{(X_o)} \quad 3.$$

The carrier conservation equation:

$$T = (P_o) + (PX_o) + (P_i) + (PX_i) \quad 4.$$

According to assumption 2, both forms of the carrier move at the same rate. Thus the number of carriers moving inward must equal those moving outward, whether a complex or not. This leads to the steady state equation:

$$D_{px}(PX_0) + D_p(P_0) = D_{px}(PX_i) + D_p(P_i) \quad 5.$$

and since $D_p = D_{px}$

$$\frac{T}{2} = (P_0) + (PX_0) = (P_i) + (PX_i) \quad 6.$$

Thus, by substituting equation 3 into 6, we can solve for (PX_0) and (PX_i) , which can be introduced into equation 1 to give

$$\frac{dx}{dt} = -V_{\max} \left(\frac{(X_0)}{K_x + (X_0)} - \frac{(X_i)}{K_x + (X_i)} \right) \quad 7.$$

Where, V_{\max} = maximum velocity = $D_{px} \cdot \frac{T}{2}$

Measurement of the parameters, K_x and V_{\max} , can be effected if the cells are preloaded with sugar, so that $(X_i) \gg K_x$

and

$$\frac{(X_i)}{K_x + (X_i)} \approx 1$$

Equation 7 then becomes,

$$v = \frac{dx}{dt} = -V_{\max} \left(\frac{(X_0)}{K_x + (X_0)} - 1 \right) \quad 8.$$

$$\frac{1}{v} = \frac{(X_0)}{V_{\max} \cdot (K_x)} + \frac{1}{V_{\max}} \quad 9.$$

Thus a plot of $1/v$ against (X_0) should give $1/V_{\max}$ as the intercept on the y axis and $-K_x$ as the intercept on the x axis. From equation 9 it can be seen that when $v = V_{\max}/2$,

$K_x = (X_0)$. The parameter, K_x , is more conveniently referred to as a half-saturation constant¹²⁷ and is kinetically different from the Michelis-Menten constant in enzyme kinetics.

CHAPTER 11

MATERIALS AND METHODS

Reagents

D-glucose (B.D.H.),

4-deoxy-4-fluoro-D-glucose and 4-deoxy-D-glucose (see part 1)

Human erythrocyte preparation

Fresh or recently outdated whole blood was obtained from the Windsor Red Cross Society, Windsor, Ontario. The blood could be stored in this condition for up to 3 weeks at 4⁰.

The cells were separated from 10 ml blood by centrifugation (3000 rpm for 10 minutes at 20⁰) and the plasma and the top layer of cells removed. They were washed twice and resuspended in saline (1% w/v NaCl-4mM sodium phosphate, PH 7.4). Washed, packed erythrocytes (1 part) were suspended in a solution of 100 mM sugar (19 parts) and incubated at 37⁰ for 30 minutes to achieve equilibrium.

Monosacharide transport

A 3 ml cuvette containing 2.5 ml of saline-buffer solution, which was either test sugar free or contained up to 50% of the pre-loaded sugar concentration in the erythrocyte cell preparation, was placed in the sample compartment of a Beckman ACTA MVI UV/VIS recording spectrophotometer. The content of the cuvette was stirred with a built-in magnetic stirrer and the temperature was maintained at 37⁰ ± 0.1 by a Forma-

Temperature bath and circulator. A portion (20 μ l) of the sugar pre-loaded erythrocyte suspension was added and the slit-width control on the spectrophotometer adjusted to bring the E_{700} on the scale 0-0.1. The absorbance scale was calibrated with the cellular concentration of sugar by equating the change in absorbance between zero time and osmotic equilibrium with the known change in internal cell concentration of sugar. Since the increase in absorbance at 700 nm can be equated with the exit time, K_x and V_{max} may be obtained from the rate equation of Miller¹²⁶.

$$\text{Exit time} \propto \frac{1}{v} = \frac{X_o}{K_x \cdot V_{max.}} + \frac{1}{V_{max}}$$

CHAPTER 111

Results And Discussion

In general the parameters of hexose transport in the human erythrocyte can be determined by one of two methods, 1) by measuring the flux of a labelled sugar, or 2) by measuring the change in volume of the cells as osmotic equilibrium is maintained during the transport of the sugar. Although the absolute values of the parameters vary according to the method used, the relative values are unaffected. The optical method was chosen because of its simplicity and convenience.

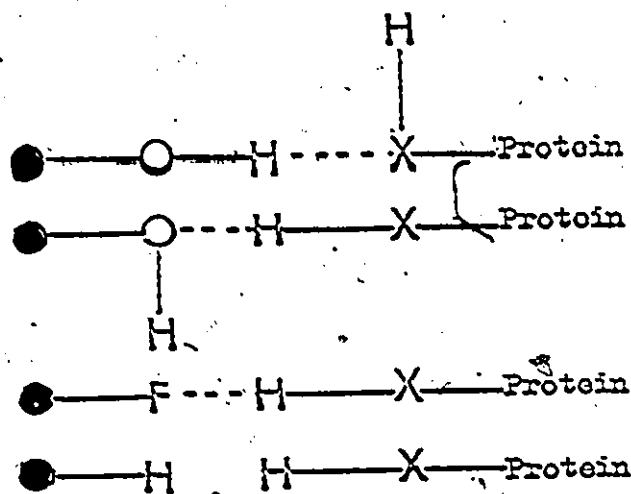
The photometric method, originally devised by Brag and Orskov¹³⁵, depends on the scattering of a beam of light by the red cells. Since cells act as osmometers, they react rapidly to changes in solute concentration, and consequently this is related to cell volume. As erythrocyte volume alters, so the amount of light scattered from a beam passing through a cell suspension changes. The change of optical density is directly related to the flux of solute across the cell membrane.

The method described by Sen and Widdas¹⁶⁶, depends on following the change in cell volume, accompanying the exit of sugar from red cells preloaded with solute, into dilute solutions of that solute. The modified rate equation of Miller¹²⁶, allows a linear plot of $1/v$ against the external concentration of sugar to give $1/V_{\max}$ as the intercept on the y axis and $-K_x$ as intercept on the x axis, where K_x is a half-

saturation constant for the sugar carrier complex. This constant may be considered as a measure of the binding affinity of sugar for the carrier system analogous to the K_m value in Michaelis-Menten kinetics. Thus comparatively low and high K_x values indicate high and low affinity binding respectively.

The idea of multipoint contact between glucose and protein through hydrogen bonding was introduced some time ago¹⁶⁷, and some of the first studies used to explore the specificity of this binding to an enzyme using halogenated sugar derivatives as probes were carried out by Helferich *et al*¹⁶⁸. Since the hydrogen bond between an -OH group of the sugar and a protein may use either the -OH group of the sugar or that of the receptor group (X) in the protein to form the hydrogen bridge (figure 39), then it follows that since fluorine can only hydrogen bond in one direction, deoxyfluorosugars may be used to probe the location and direction of hydrogen bonding between the sugar and protein. Thus the presence of a hydrogen bond at each position in the sugar may be investigated by comparing the kinetic transport (or inhibition) constants for sugar in which the hydroxyl group has been replaced by fluorine or hydrogen. If the presence of the oxygen of the hydroxyl group is necessary for hydrogen bonding then stereospecific substitution by fluorine should reveal only minor change in the half saturation (K_x) (or inhibition, K_i) constant. Similarly, substitution of this hydroxyl group by hydrogen, should prevent this type of bonding and be reflected by an increase in K_x (or K_i) value

(i.e. a decrease in binding affinity).



**Figure 39 POSSIBLE HYDROGEN BONDS BETWEEN HYDROXYL, FLUORINE
SUBSTITUTED SUGARS (O). AND PROTEIN (X = O, S AND N)**

The results obtained for the exit of D-glucose, 3-deoxy-3-fluoro-D-glucose and 3-deoxy-D-glucose¹⁶⁹ (table 2) indicate that replacement of the oxygen function at C₃ of D-glucose by fluorine does not significantly change the K_x value or V_{max} for the carrier protein. In contrast 3 deoxy-D-glucose has lost this ability to hydrogen bond at C₃ and consequently has a lower affinity for carrier protein (higher K_x value). The result with 5-thio-D-glucose¹¹⁷ (Table 2) in which the ring oxygen at C₅ in D-glucose has been replaced by sulphur is included to illustrate the importance of the ring oxygen as a possible hydrogen bonding site. Using fluorinated glucoses

TABLE 2

Exit Transport Parameters of Monosaccharides Across Human Erythrocyte Membrane at 37°C.

| Suger | K_x (mM) | v_{\max} (mmol. lit ⁻¹ min ⁻¹) |
|----------------------------|------------|--|
| D-glucose | 3.9 | 640 ^a |
| 3-deoxy-3-fluoro-D-glucose | 2.3 | 600 ^a |
| 3-deoxy-D-glucose | 15.3 | 795 ^a |
| 5-Thio-D-glucose | 15.0 | 500 ^b |

a, from Re f. 169; b, from Ref. 117

as probes but by an entirely different method, extensive studies by Barnett et al¹⁷⁰ have demonstrated the importance of oxygen functions at C₁ (β -orientation) and C₃ in glucose for binding to the carrier. Thus a comparison of the inhibition of the poorly transported L-sorbose entry in the human erythrocyte by D-glucose, deoxyfluoro- and deoxy-D-glucose analogues may be considered to reflect the affinity of the sugars for the carrier system. For example, 1-deoxy-D-glucose and α -D-glucosyl fluoride were poor competitive inhibitors (relatively high K_i values) Whereas β -D-glucosyl fluoride had a K_i value closer to D-glucose (Table 3) which suggests the importance of a β -oriented hydroxyl group at C₁ of glucose. Likewise the K_i values for 3-deoxy-D-glucose and 3-deoxy-3-fluoro-D-glucose implicate the oxygen function at C₃ in hydrogen bonding. Unlike the case of glucose transport in hamster intestine, the oxygen function at C₆ does not appear to be involved in hydrogen bonding since there is no major change in the K_i values for 6-deoxy-D-glucose and 6-deoxy-6-fluoro-D-glucose.

The function of the C₄-hydroxyl group has been controversial^{170,171}. Since one of the objectives of the present programme was to find out the importance of the oxygen function at C₄ for hydrogen bonding with carrier protein, D-glucose, 4-deoxy-4-fluoro-D-glucose and 4-deoxy-D-glucose were used for a kinetic study using the Sen and Widdas method¹⁶⁶. The results obtained for the exit of D-glucose (figure 40), 4-deoxy-4-fluoro-D-glucose (figure 41) and 4-deoxy-D-glucose (figure 42) (Table 4)

TABLE 3

Inhibition of L-sorbose Entry into Human Erythrocytes by
Glucose Analogues.

| Sugar | K_i mM, 25°C |
|-------------------------------|----------------|
| Glucose | 7.6 |
| 1-Deoxyglucose | 76.0 |
| α -D-glucosyl fluoride | 77.0 |
| β -D-glucosyl fluoride | 15.4 |
| 3-Deoxyglucose | 71.5 |
| 3-Deoxy-3-fluoro-glucose | 6.9 |
| 6-Deoxyglucose | 6.7 |
| 6-Deoxy-6-fluoro-glucose | 1.2 |

For experimental details see ref. 170.

Figure 40

DETERMINATION OF THE KINETIC PARAMETERS OF D-GLUCOSE AT

$37.0 \pm 0.1^{\circ}$

$K_x = 4.0 \text{ (mM)},$

$V_{\max} = 645 \text{ mmol lit}^{-1} \text{ min}^{-1}.$

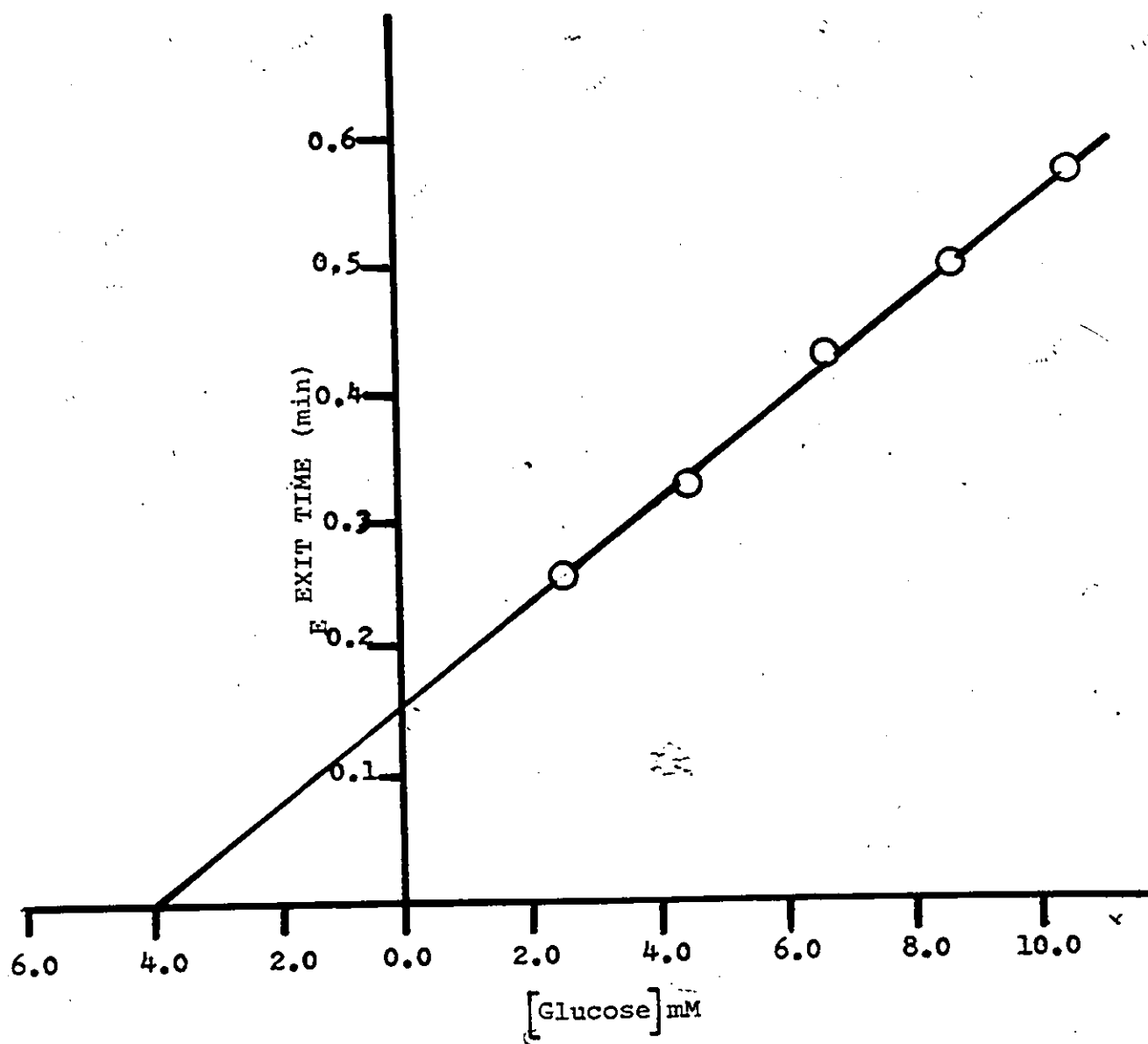


Figure 41

DETERMINATION OF THE KINETIC PARAMETERS OF 4-DEOXY-4-
FLUORO-D-GLUCOSE AT $37.0 \pm 0.1^\circ$

$$K_x = 4.6 \text{ (mM)}$$

$$V_{\max} = 645 \text{ mmol lit}^{-1} \text{ min}^{-1}$$

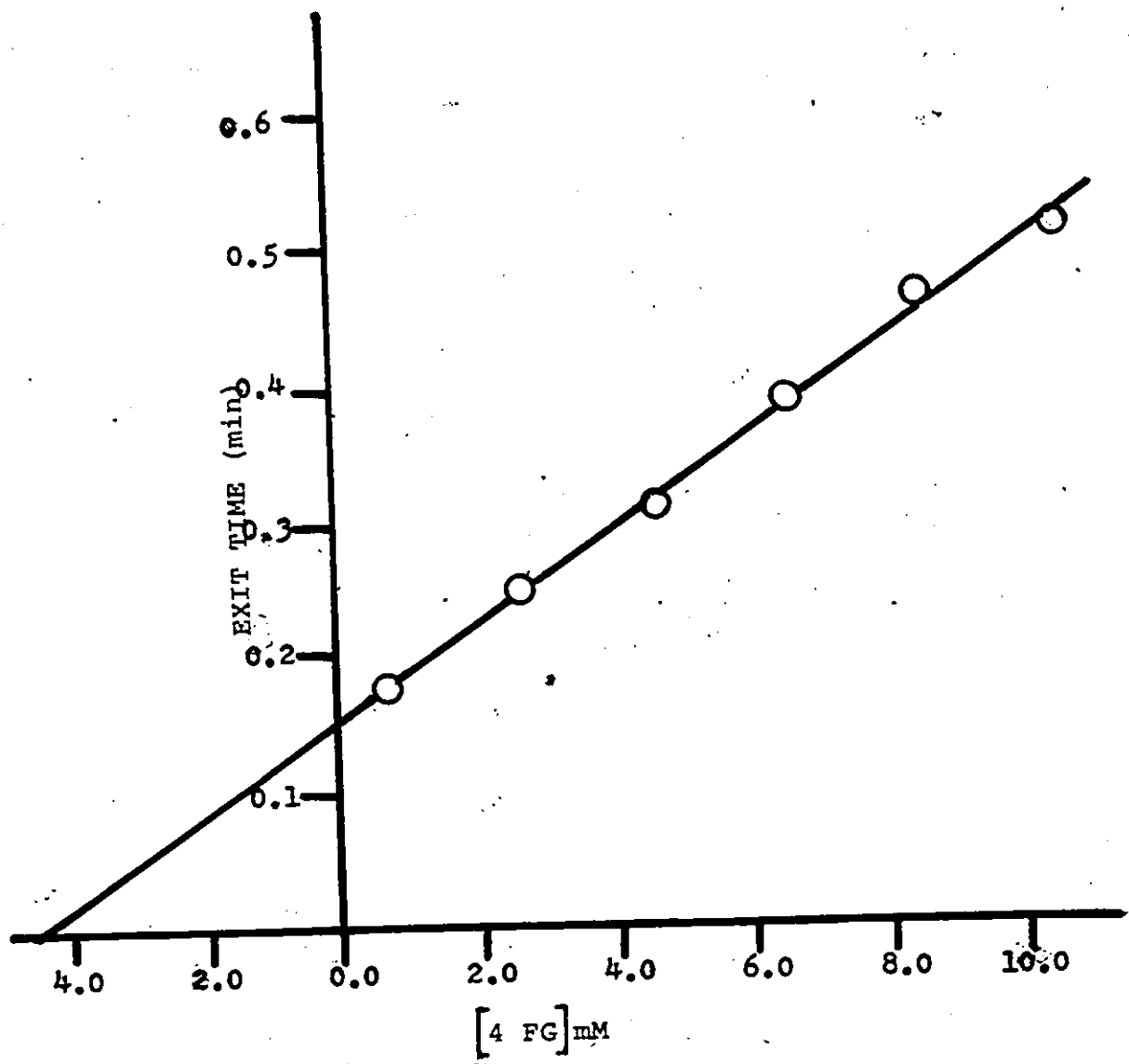


Figure 42

DETERMINATION OF THE KINETIC PARAMETERS OF 4-DEOXY-D-
GLUCOSE AT $37.0 \pm 0.1^{\circ}$

$K_x = 4.5 \text{ (mM)}$; $V_{\text{max}} = 645 \text{ mmol lit}^{-1} \text{ min}^{-1}$

○ = Duplicate Expt.

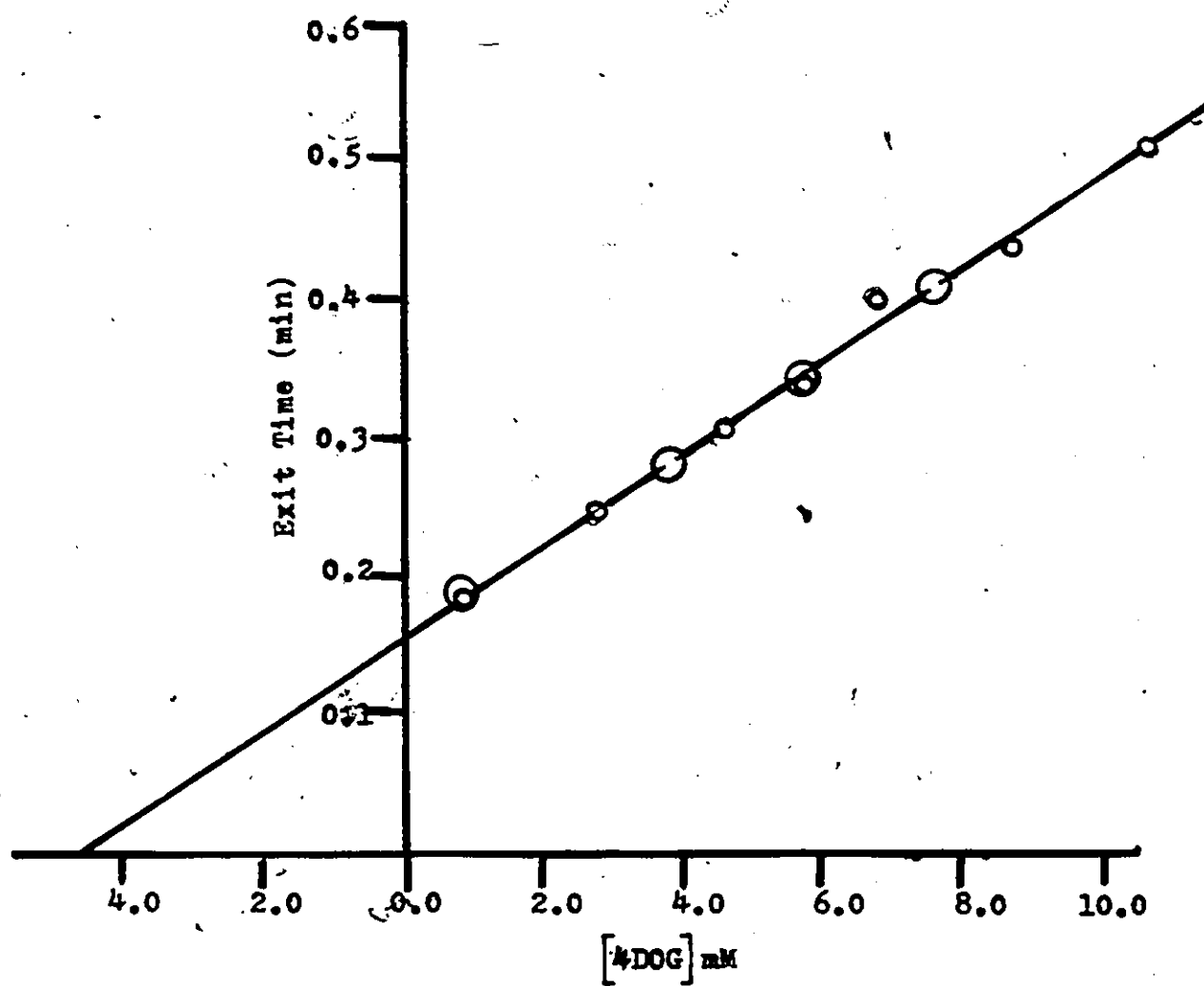


TABLE 4

Exit Transport Parameters of Monosaccharides Across Human
Erythrocyte membrane at 37°C

| Sugar | K_x | V_{max} (mmol. lit ⁻¹ min ⁻¹) |
|--------------------------------|-------|---|
| Glucose | 4.0 | 645 a |
| 4-deoxy-4-fluoro- D-glucose | 4.60 | 645 b |
| 4-deoxy-D-glucose | 4.50 | 645 c |

a = from figure 40

b = from figure 41

c = from figure 42

indicate that replacement of oxygen function at C_4 by either fluorine or hydrogen does not significantly change the K_x value for the carrier protein. Therefore C_4 -OH group of the D-glucose is probably not involved in hydrogen bonding with carrier protein.

Unfortunately a systematic study to elucidate the importance of the oxygen function at C_2 has not been done and therefore the importance of the oxygen function at C_2 for hydrogen bonding remains debatable. However results of Kahlenberg and Dolansky¹⁷¹ suggest that oxygen function at C_2 of D-glucose is probably not involved in hydrogen bonding with carrier protein. The method used by these workers was entirely different from the present one. In their experimental situation, the binding of a D-glucose analogue was assessed by its ability to inhibit the stereospecific uptake of D-(³H)-glucose from the cell ghosts¹⁷¹. The fact that 2-deoxy-D-glucose competitively inhibits the uptake of D-(³H)-glucose to the same extent as D-glucose, suggested that C_2 oxygen function of D-glucose is probably not involved in hydrogen bonding with carrier protein.

Taken together, these studies with fluorinated sugars constitute further evidence for the existence of at least three stereospecific hydrogen bonding between oxygen atoms at C_1 , C_3 and the ring oxygen at C_5 of β -D-glucopyranose and the carrier protein (Figure 43).

These results also agree with the proposed model¹⁷² for the inhibition of D-glucose transport in human erythrocytes

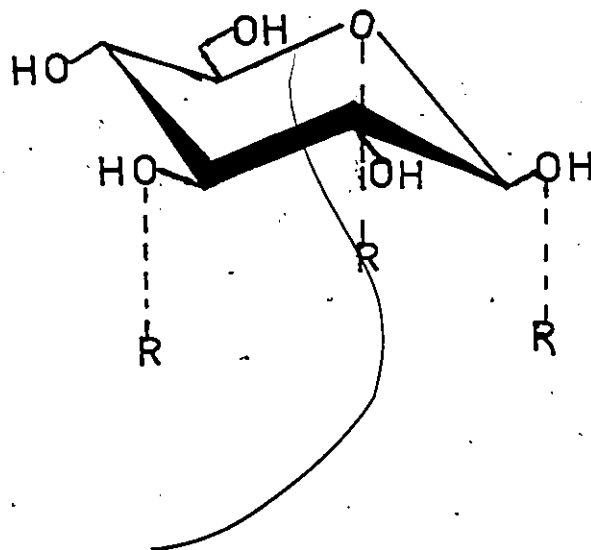
by Cytochalasin B and the detection of three different receptor groups ($-\text{NH}_2$, $-\text{SH}$, and imidazole) on the protein associated with D-glucose transport in the human erythrocyte¹⁵⁸.

Figure 43

 β -D-GLUCOPYRANOSE C1-CONFORMATION

R = PROTEIN RECEPTOR GROUPS

----- HYDROGEN BONDS



APPENDIX

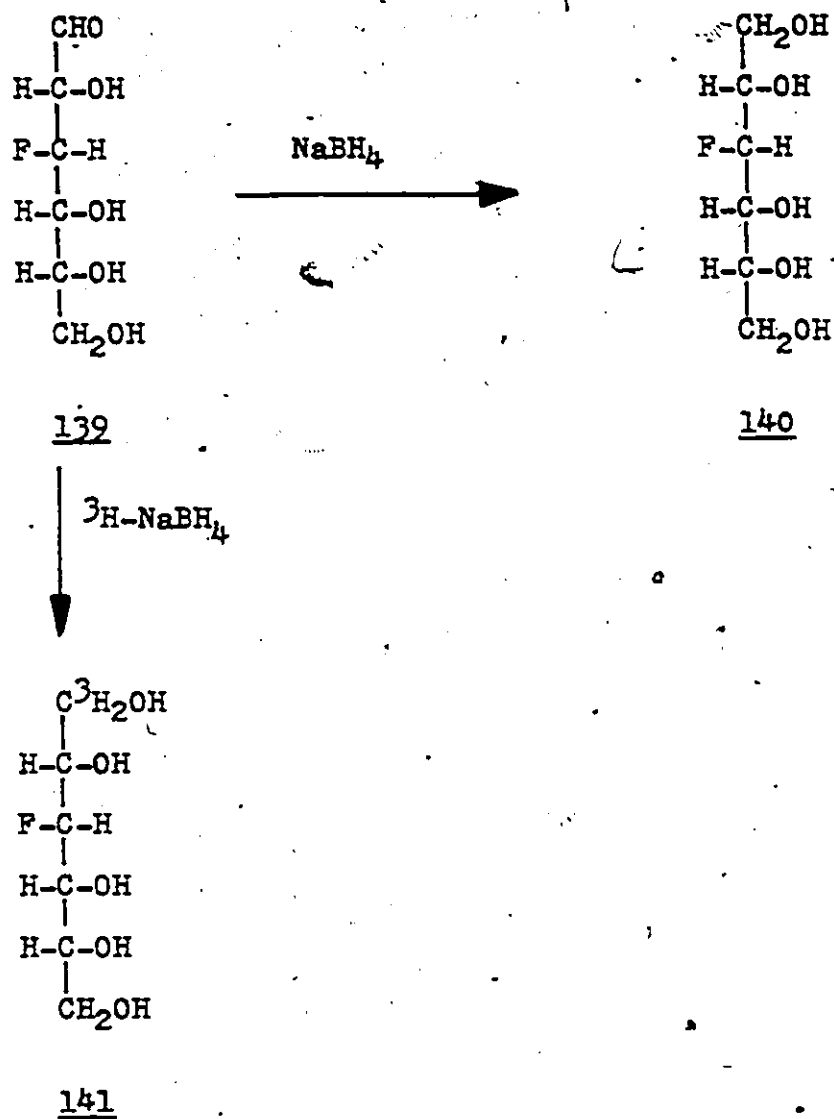


Figure 44. SYNTHESIS OF 3-DEOXY-3-FLUORO-D-GLUCITOL AND
 $^3\text{H}-(\text{C}_1)$ -3-DEOXY-3-FLUORO-D-GLUCITOL.

3-deoxy-3-fluoro-D-glucitol (140).

To 3-deoxy-3-fluoro-D-glucose⁴³ (500 mg) in water (10 ml) was added dropwise a solution of sodium borohydride (500 mg) in water (15.0 ml) with stirring. The temperature was not allowed to exceed 50°. After standing 3 hours Amberlite IR-120 (H⁺) was added until the evolution of gas ceased. The mixture was diluted with water (50 ml) and stirred for 15 minutes with an addition 2.5 ml of Amberlite IR-120 (H⁺). After filtration, the filtrate was evaporated in vacuo to a syrup which was repeatedly dissolved in methanol and evaporated to dryness in vacuo to remove methyl borate. The resulting syrup was dissolved in minimum amount of methanol. The addition of petroleum ether (b.p. 30-60) yielded a non-reducing (negative test with Fehling's solution and aniline phthalate spray) crystalline 3-deoxy-3-fluoro-D-glucitol (450 mg) which recrystallised from methanol-petroleum ether (b.p. 60-80). m.p. 121°, (α)_D²³ - 9° (c 4.00, water), R_F 0.55 (solvent N), 0.66 (solvent M). Molecular peak at 184 (M⁺) and 185 (M⁺ + 1) in field desorption mass spectrum. Anal. calc. for C₆H₁₃O₅F: C, 39.1; H, 7.1; F, 10.3
found: C, 39.1; H, 7.1; F, 9.9

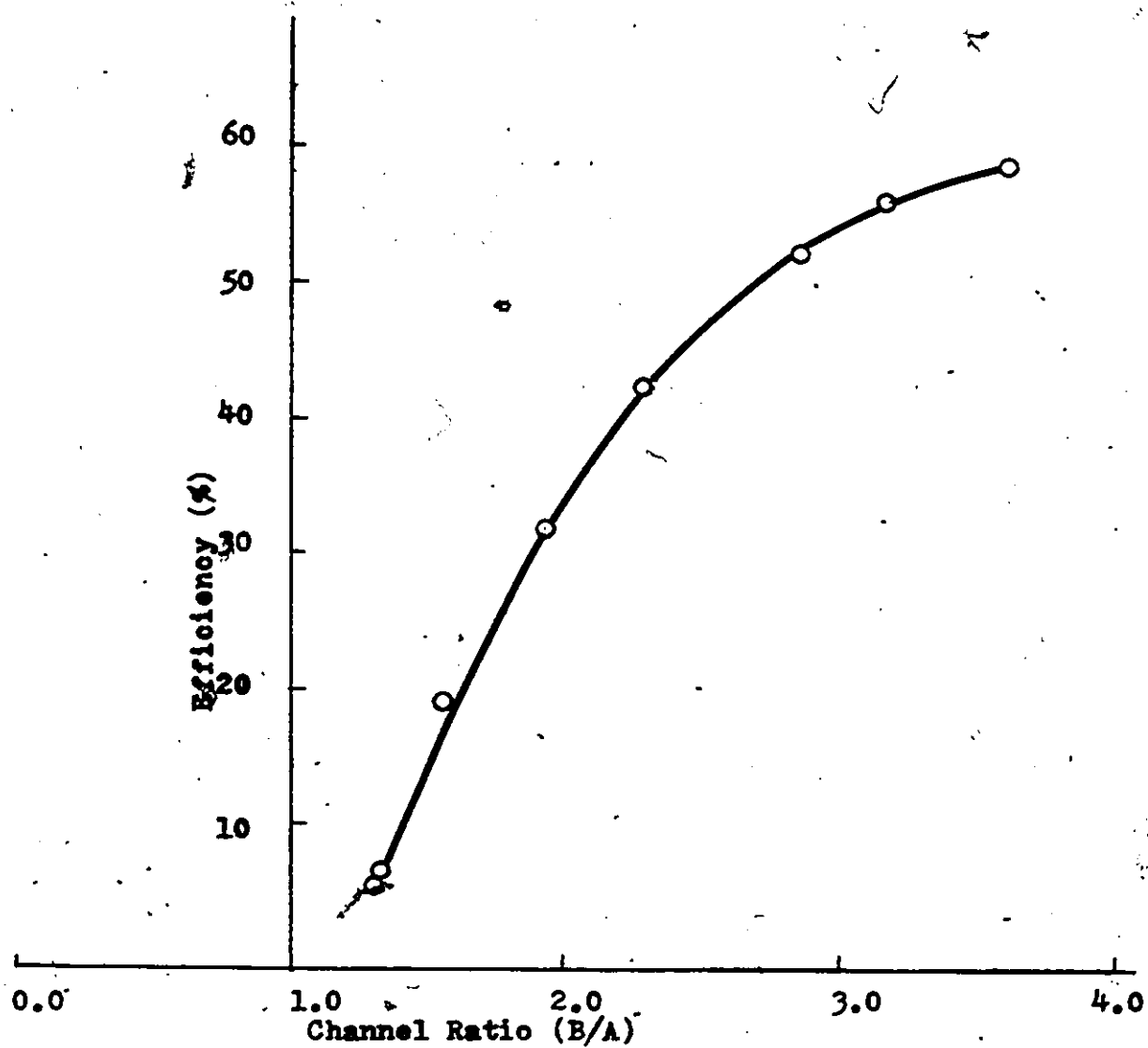
$^3\text{H}-(\text{C}_1)$ -3-deoxy-3-fluoro-D-glucitol (141)

To an ice-cold solution of 3-deoxy-3-fluoro-D-glucose (182 mg in 3 ml of water) was added dropwise, under magnetic stirring, an ice-cold solution of ^3H -sodium borohydride (25 mci). After standing for two hours at room temperature the mixture was treated with excess sodium borohydride, stirred two more hours and diluted with 15 ml of water. Amberlite IR-120(H^+) was added until the evolution of gas stopped, and stirred for 15 minutes. After filtration, the filtrate was evaporated to a thin syrup. The syrup was repeatedly dissolved in methanol and evaporated at reduced pressure to remove boric acid as methyl borate. The resulting syrup was dissolved in minimum amount of methanol. The addition of petroleum ether (b.p. 30-60) yielded a non-reducing (negative test with aniline phthalate spray) crystalline $^3\text{H}-(\text{C}_1)$ -3-deoxy-3-fluoro-D-glucitol which was recrystallised from methanol-petroleum ether (b.p. 60-90) (162 mg). m.p. 121° , R_F 0.55 (solvent N), 0.66 (solvent M). Radiochemical yield 45.4%. (Sp. activity 12.8 mci/mole, total activity 11.3 mci).

Figure 45

DETERMINATION OF A EFFICIENCY OF THE TRITIUM SAMPLES,
USING THE STANDARD CURVE.

| Compounds | Efficiency |
|--|------------|
| $^3\text{H}-(\text{C}_3)-1,2;5,6\text{-di-}\underline{\text{O}}\text{-isopropylidene-}$ $3\text{-}\underline{\text{O}}\text{-toluene-p-sulphonyl-}\alpha\text{-D-}$ allofuranose (135) | |
| First crop | 40.05% |
| Second crop | 39.55% |
| $^3\text{H}-(\text{C}_3)-3\text{-deoxy-3-fluoro-}\alpha,\beta\text{-D-}$ glucose (138) | 32.00% |
| $^3\text{H}-(\text{C}_1)-3\text{-deoxy-3-fluoro-D-}$ glucitol (141) | 36.00% |



Calculation for determination of radioactivity of tritium samples

A plot of B/A (channel ratio) against efficiency for standard tritium samples is shown in Figure 45.

$$\text{Efficiency} = \frac{\text{CPM on channel A} + \text{CPM on channel B}}{\text{DPM}}$$

An efficiency for $^3\text{H}-(\text{C}_3)$ -3-deoxy-3-fluoro-D-glucose (138) $^3\text{H}-(\text{C}_3)$ -1,2;5,6-di-O-isopropylidene-3-O-toluene-p-sulphonyl- α -D-allofuranose (135) and $^3\text{H}-(\text{C}_1)$ -3-deoxy-3-fluoro-D-glucitol (141) was calculated from Figure 45.

(A) $^3\text{H}-(\text{C}_3)$ -1,2;5,6-di-O-isopropylidene-3-O-toluene-p-sulphonyl- α -D-allofuranose

First crop

$$3.45 \times 10^{-6} \text{ g} = 4,476 \text{ CPS}$$

$$2.616 \text{ g} = 91.62 \text{ mci}$$

Second crop

$$14 \times 10^{-6} \text{ g} = 5,371 \text{ CPS}$$

$$0.271 \text{ g} = 2.8 \text{ mci}$$

$$\text{Total activity } 91.62 + 2.8 = 94.42 \text{ mci}$$

(B) $^3\text{H}-(\text{C}_3)$ -3-deoxy-fluoro-D-glucose

$$2.65 \times 10^{-6} \text{ g} = 7,896 \text{ CPS}$$

$$0.678 \text{ g} = 54.6 \text{ mci}$$

$$\text{Sp. Activity} = \frac{54.6}{678} \times 182 = 14.7 \text{ mci/mmole}$$

c) $^3\text{H}-(\text{C}_1)-3\text{-deoxy-3-fluoro-D-glucitol}$

$$0.56 \times 10^{-6} = 31,407 \text{ CPM}$$

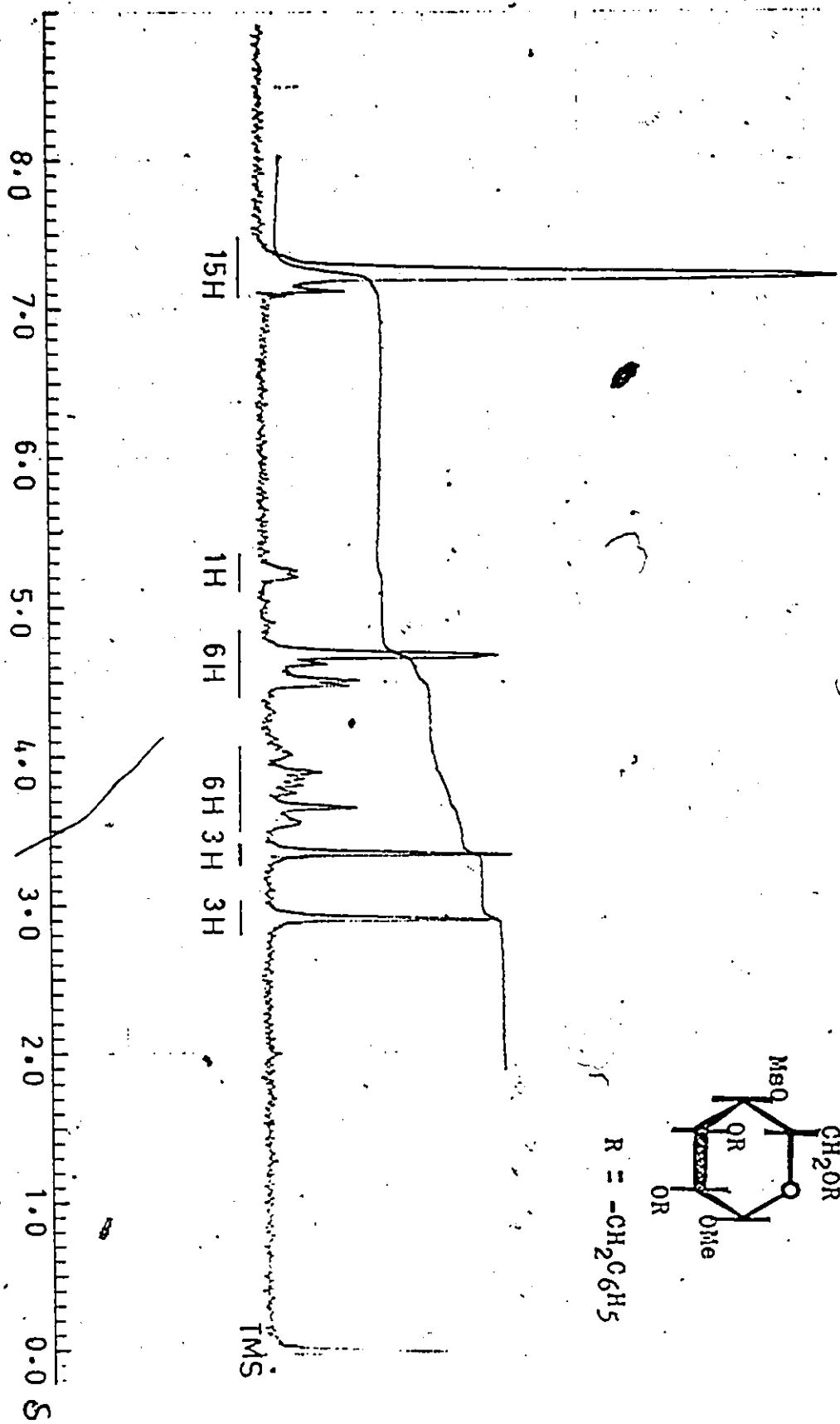
$$0.162 \text{ g} = 11.3 \text{ mci}$$

$$\text{Sp. Activity} = \frac{11.3}{162} \times 184 = 12.8 \text{ mci/mole}$$

Figure 46

N.M.R. SPECTRUM OF METHYL 2,3,6-TRI-O-BENZYL-4-O-METHYL-SULPHONYL - α -D-GALACTOPYRANOSIDE.

7.25(s, 15H, C₆H₅), 5.25(d, J = 2.5, 1H, C₁H), 4.75-4.45
(m, 6H, CH₂C₆H₅), 4.1-3.5(m, 6H, C₂H, C₃H, C₄H, C₅H, C₆H₂),
3.35(s, 3H, -OCH₃), 2.9(s, 3H, -SO₃-CH₃)



R = $-\text{CH}_2\text{C}_6\text{H}_5$

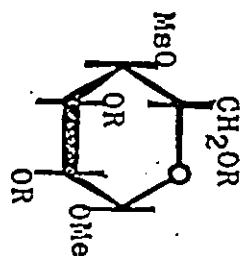


Figure 47

METHYL 2,3,6-TRI-O-BENZYL-4-DEOXY-4-FLUORO- α -D-GLUCOPYRANO-
SIDE. (118)

-7.3 (s, 15H, $-\text{C}_6\text{H}_5$), 5.05-3.5 (m, 13H, C_1H , C_2H , C_3H , C_4H , C_5H , C_6H_2 , $\text{CH}_2-\text{C}_6\text{H}_5$), 3.4 (s, $-\text{OCH}_3$)

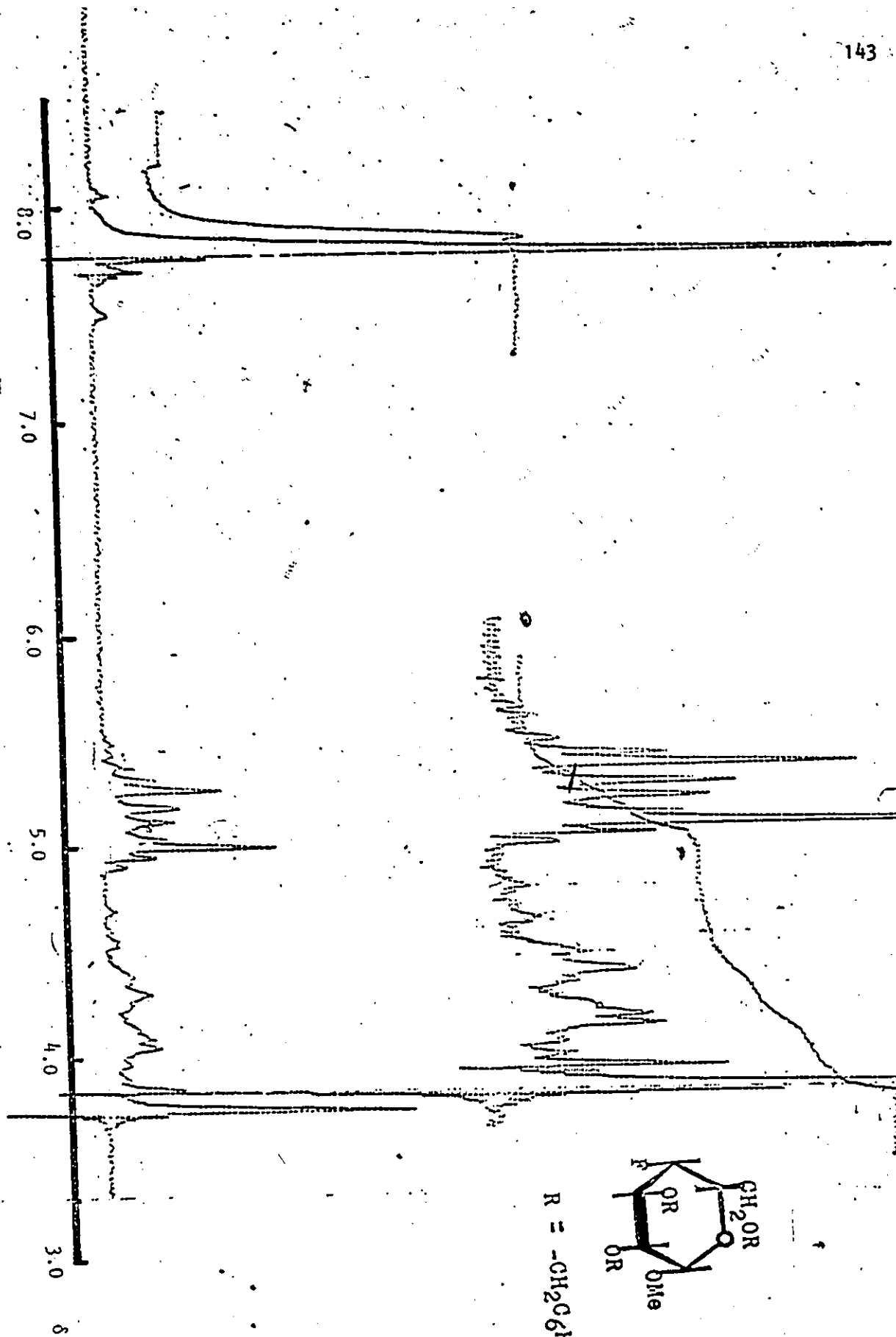


Figure 48

N.M.R. SPECTRUM OF METHYL 2,3,6-TRI-O-ACETYL-4-DEOXY-4-
FLUORO- α -D-GLUCOSPYRANOSIDE (121) IN C_6D_6 .

5.4-6.0 (1H, $J_{4,5} = J_{4,3} = 9.5$ Hz, $J_{4,8} = 3.75$, C_4H),
4.65(t, 1H, C_1H), 4.6-4.1(cm, 2H, C_6H_2), 4.1-3.9(cm, 1H, C_5H),
3.7(t, $J = 9.0$ Hz, C_3H), 3.55-3.3(cm, 1H, C_2H), 2.65(s, 3H,
-OCH₃), 1.45(d, $J = 4.5$, 9H, $\begin{smallmatrix} \text{---C---CH}_3 \\ || \\ \text{O} \end{smallmatrix}$)

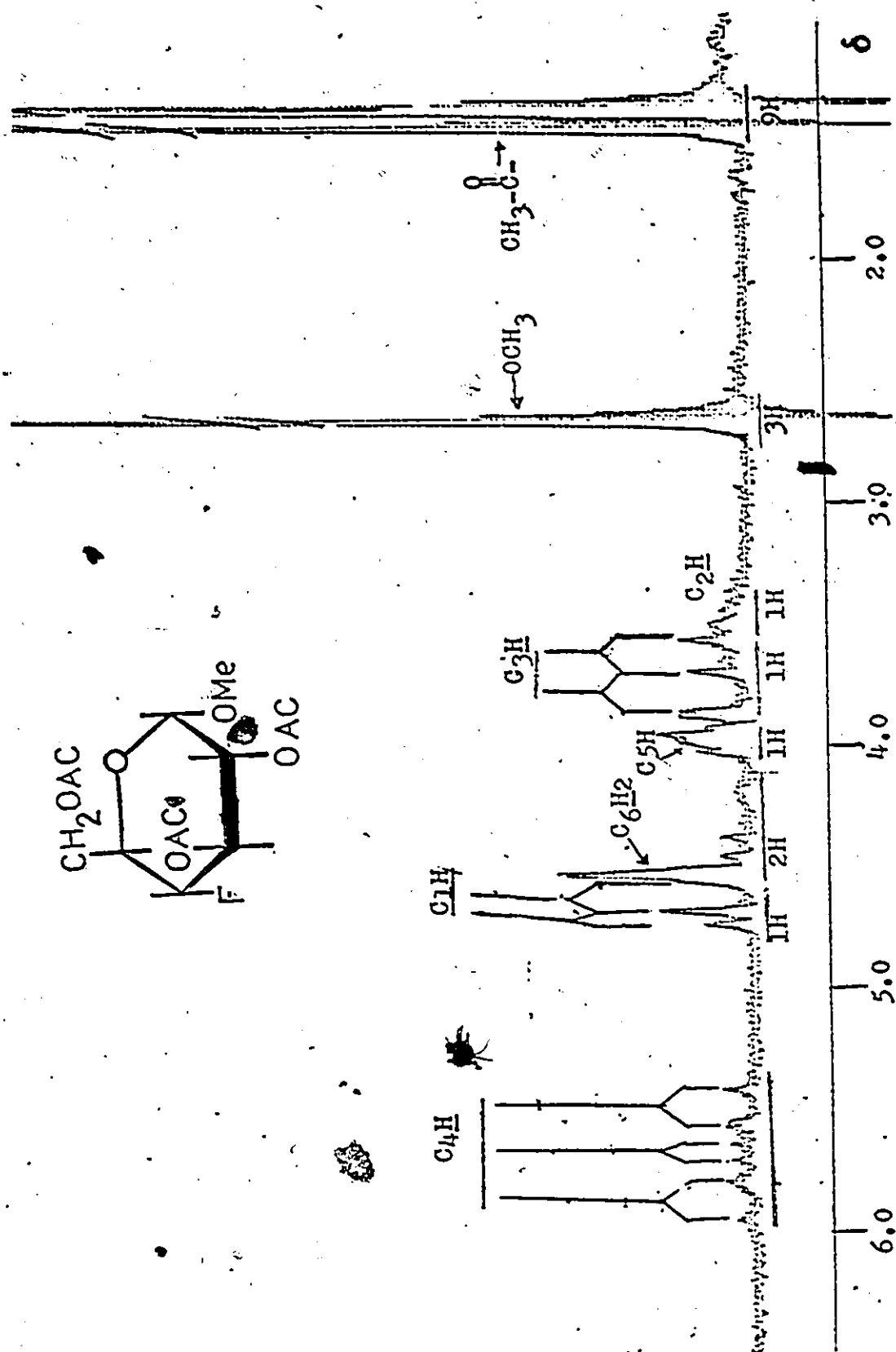


Figure 49

METHYL 2,3,6-TRI-O-BENZOYL-4-DEOXY-4-iodo- α -D-GLUCOPYRANOSIDE (122) IN CDCl_3 .

8.3-7.25(m, 15H, $-\text{C}_6\text{H}_5$), 6.2(t, J = 9.5 Hz, C_4H),

5.3(s, 1H, C_1H), 5.15(t, 1H, $J_{2,1} = 4\text{Hz}$, $J_{2,3} = 5\text{Hz}$, C_2H),

5-4.1(cm, 4H, C_3H , C_5H , C_6H_2), 3.5(s, 3H, $-\text{OCH}_3$).

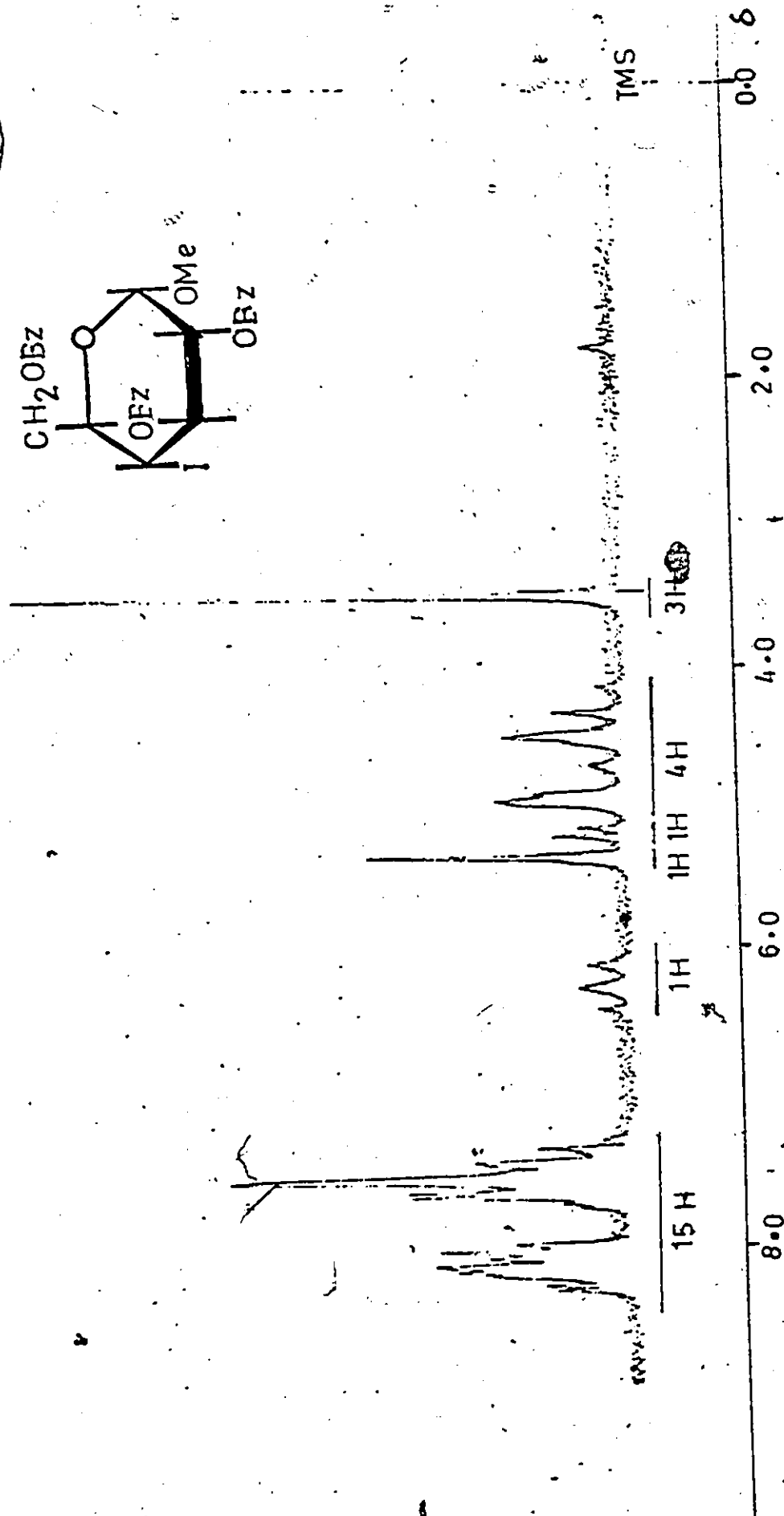


FIGURE 50

N.M.R. SPECTRUM OF METHYL 2,3,6-TRI-O-BENZOYL-4-DEOXY-4-
IODO- α -D-GALACTOPYRANOSIDE (132) IN CDCl_3

8.3-7.2(m, 15H, C_6H_5), 6.4-4.9(cm, 7H, C_1H , C_2H , C_3H , C_4H ,
 C_5H , C_6H_2), 3.5(s, 3H, $-\text{OCH}_3$)

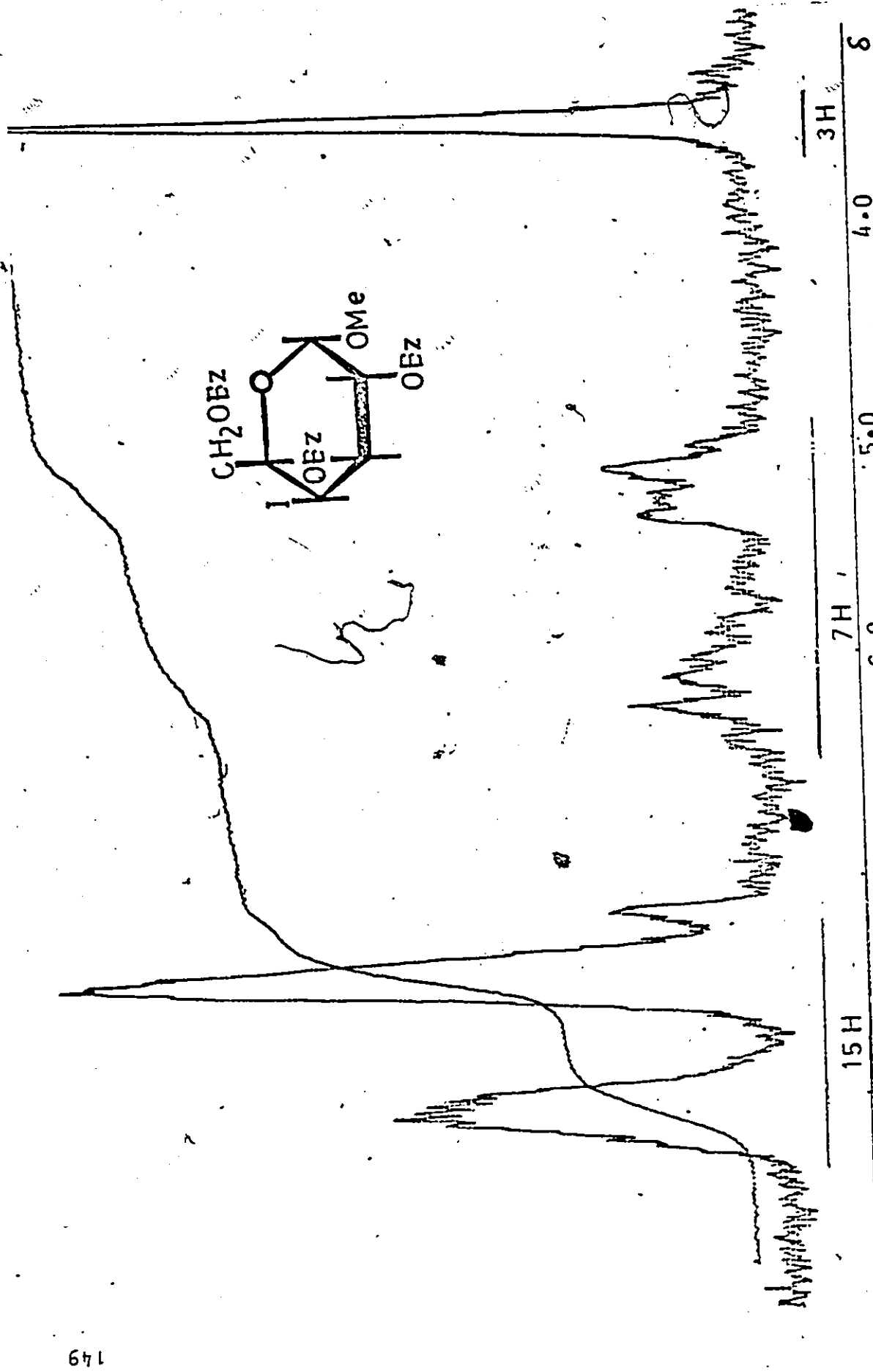


Figure 51

T.L.C. PLATE 1 AND 2:

- 1 = 4-deoxy-4-fluoro-D-glucose.
- 2 = Reaction mixture.
- 3 = 3-deoxy-3-fluoro-D-arabinose.



PLATE #1

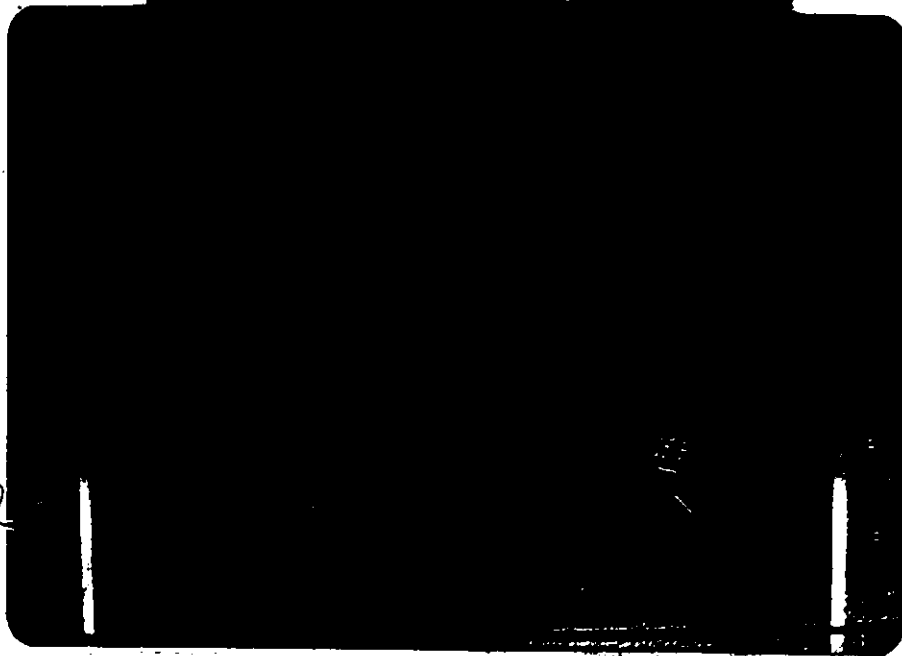


PLATE #2

COLOURER PHOTO

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